DOES NOT CIRCULATE

VOLUME IV NUMBER 2 & 3

DEC 1960
MEDICAL LIBRARY

# THE JOURNAL OF EXFOLIATIVE CYTOLOGY

*The Official Periodical of*
THE INTERNATIONAL ACADEMY OF GYNECOLOGICAL CYTOLOGY
*Organe Officiel de*
L'ACADEMIE INTERNATIONALE DE CYTOLOGIE GYNECOLOGIQUE
*Das Offizielle Organ der*
INTERNATIONALEN AKADEMIE FÜR GYNÄKOLOGISCHE ZYTOLOGIE
*Organo Oficial de*
LA ACADEMIA INTERNACIONAL DE CITOLOGIA GINECOLOGICA
*and*
*the Official Periodical of*
THE INTER-SOCIETY CYTOLOGY COUNCIL

The Journal is also the Official Publication of the Following Societies:

SOCIEDADE BRASILEIRA DE CITOLOGIA
(*Orgão Oficial*)

DEUTSCHE GESELLSCHAFT FÜR ANGEWANDTE ZYTOLOGIE
(*Offizielles Organ*)

SOCIETÀ ITALIANA DI CITOLOGIA CLINICA E SOCIALE
(*Organo Ufficiale*)

ASOCIACION MEXICANA DE CITOLOGIA EXFOLIATIVA
(*Organo Oficial*)

*Honorary Editor:* GEORGE N. PAPANICOLAOU *Editor:* GEORGE L. WIED

*Editorial Board:*
CLARICE AMARAL FERREIRA, RUTH M. GRAHAM, EMMERICH von HAAM,
J. PAUL PUNDEL, JAMES W. REAGAN

*National Editors:* NILO P. LUZ (Brasil), PETER STOLL (Deutschland), LUIGI CUSMANO (Italia),
ALEXANDER MEISELS (México)

*Editorial Advisory Board:* see inside page

*itorial Office:* 5841 SOUTH MARYLAND AVENUE • CHICAGO 37 • ILLINOIS • U.S.A.

# EDITORIAL COMMUNICATIONS

**PUBLICATIONS.** Articles published in ACTA CYTOLOGICA are:

(1) Review articles on special phases of exfoliative cytology
(2) Original publications on all phases of exfoliative cytology
(3) Symposia by Correspondence or
(4) Papers or communication which are published as Letters to the Editors.

**MANUSCRIPTS.** Manuscripts should be *typewritten* on one side of the paper only, with double spacing and liberal margins. Articles will be published in English and authors *are requested to submit their manuscripts in English*, but, if necessary, French, German, and Spanish manuscripts will be accepted. Authors will receive a typewritten copy of the English translation for their approval. Papers submitted in French, German or Spanish, belonging to portions of the ACTA CYTOLOGICA for which a *deadline* for receipt of the papers is given, however, should REACH the Editorial Office 30 days prior to the deadline for papers in the English language, to permit translation and subsequent approval by the author.

**LETTERS TO THE EDITORS.** Discussions are invited on any subject treated in previous editions of ACTA CYTOLOGICA. Contributions may also be published if they treat other subjects than those discussed by previously published papers, if they are of general interest.

**BIBLIOGRAPHY.** References should be placed *at the end of the article* and should conform to the style of the Quarterly Cumulative Index Medicus: viz., author, periodical, volume, page and year.

**ILLUSTRATIONS.** Illustrations accompanying manuscripts should be numbered, provided with suitable legends and marked lightly on the back, with the author's name. Photographs should be supplied only on good glossy photographic paper. The minimal size is 4 × 5 inches (approximately 10 × 12 cm). Every microphotograph should show a *proportional 10μ scale* on the reverse side of the picture. Authors who wish to have color plates reproduced should be prepared to defray the cost of this process. The original drawings (not the photographic reproductions thereof) should accompany the manuscript.

**REPRINTS.** Authors are charged for reprints, and orders for reprints should be received prior to publication of the particular issue of ACTA CYTOLOGICA.

**SUBSCRIPTION.** The cost of an annual subscription is $9.50. Checks should be made payable to ACTA CYTOLOGICA and sent to ACTA CYTOLOGICA, 5841 South Maryland Avenue, Chicago 37, Illinois, U.S.A.

**RESPONSIBILITY.** While manuscripts received are subject to editing so that they conform to the style of ACTA CYTOLOGICA, the journal cannot and does not assume responsibility for statements made by contributors. The programs and their sequence are subject to change without further notice.

**COMMUNICATIONS.** Contributions, letters, and all other communications relating to ACTA CYTOLOGICA should be sent to the Editorial Office, 5841 South Maryland Avenue, Chicago 37, Illinois, U.S.A.


## ACTA CYTOLOGICA

**Published triannually under the auspices of THE INTERNATIONAL ACADEMY OF GYNECOLOGICAL CYTOLOGY, in co-operation with THE UNIVERSITY OF CHICAGO and THE CANCER RESEARCH FOUNDATION.**

**Published, 1960**



**Copyright by The International Academy of Gynecological Cytology, 1960**
**Copyright under the International Copyright Union (Berne), 1960**

**Translation, reproduction and republication of articles (and portions thereof) published in ACTA CYTOLOGICA are permitted after written approval has been obtained from the Editorial Office for each article and portions thereof.**

VOLUME IV 1960 NUMBERS 2 & 3

# ACTA CYTOLOGICA

## THE JOURNAL OF EXFOLIATIVE CYTOLOGY

*The Official Periodical of*
THE INTERNATIONAL ACADEMY OF GYNECOLOGICAL CYTOLOGY
*Organe Officiel de*
L'ACADEMIE INTERNATIONALE DE CYTOLOGIE GYNECOLOGIQUE
*Das Offizielle Organ der*
INTERNATIONALEN AKADEMIE FÜR GYNÄKOLOGISCHE ZYTOLOGIE
*Organo Oficial de*
LA ACADEMIA INTERNACIONAL DE CITOLOGIA GINECOLOGICA
*and*
*the Official Periodical of*
THE INTER-SOCIETY CYTOLOGY COUNCIL

The Journal is also the Official Publication of the Following Societies:
SOCIEDADE BRASILEIRA DE CITOLOGIA
(*Orgão Oficial*)
DEUTSCHE GESELLSCHAFT FÜR ANGEWANDTE ZYTOLOGIE
(*Offizielles Organ*)
SOCIETÀ ITALIANA DI CITOLOGIA CLINICA E SOCIALE
(*Organo Ufficiale*)
ASOCIACION MEXICANA DE CITOLOGIA EXFOLIATIVA
(*Organo Oficial*)

*Honorary Editor:* GEORGE N. PAPANICOLAOU *Editor:* GEORGE L. WIED

*Editorial Board:*
CLARICE AMARAL FERREIRA, RUTH M. GRAHAM, EMMERICH von HAAM,
J. PAUL PUNDEL, JAMES W. REAGAN
*National Editors:* NILO P. LUZ (Brasil), PETER STOLL (Deutschland), LUIGI CUSMANO (Italia),
ALEXANDER MEISELS (México)

*Editorial Advisory Board for 1960:*

GENERAL POLICY AND GYNECOLOGY AND OBSTETRICS
T. ANTOINE, Vienna, Austria
W. BICKENBACH, Munich, Germany
R. BOURG, Brussels, Belgium
L. T. COGGESHALL, Chicago, Ill., USA
M. EDWARD DAVIS, Chicago, Ill., USA
P. F. DENOIX, Villejuif, France
G. DELLEPIANE, Torino, Italy
P. FUNCK-BRENTANO, Paris, France
E. HELD, Zurich, Switzerland
Th. KOLLER, Basel, Switzerland
J. V. MEIGS, Boston, Mass., USA
A. de MORAES, Rio de Janeiro, Brazil
E. NAVRATIL, Graz, Austria
H. RUNGE, Heidelberg, Germany
L. C. SCHEFFEY, Philadelphia, Pa., USA
H. de WATTEVILLE, Geneva, Switzerland

PATHOLOGY
H. BETTINGER, Melbourne, Australia
J. CAMPOS R. de C., Lima, Peru
J. DELARUE, Paris, France
H. LAX, Berlin, Germany
J. H. MÜLLER, Zürich, Switzerland
C. W. TAYLOR, Birmingham, England

ENDOCRINOLOGY
W. M. ALLEN, St. Louis, Mo., USA
J. BOTELLA LLUSIA, Madrid, Spain
J. FERIN, Louvain, Belgium
A. E. RAKOFF, Philadelphia, Pa., USA
B. ZONDEK, Jerusalem, Israel

PUBLIC HEALTH
E. DAY, New York, N.Y., USA
R. F. KAISER, Bethesda, Md., USA
K. MASUBUCHI, Tokyo, Japan

BASIC CYTOLOGY
SIR ROY CAMERON, London, England
T. O. CASPERSSON, Stockholm, Sweden
I. GERSH, Chicago, Ill., USA
G. O. GEY, Baltimore, Md., USA
A. GLÜCKSMANN, Cambridge, England
H. LETTRÉ, Heidelberg, Germany
R. C. MELLORS, New York, N.Y., USA
W. SANDRITTER, Frankfurt a. M., Germany

NON-GYNECOLOGICAL EXFOLIATIVE CYTOLOGY
P. A. HERBUT, Philadelphia, Pa., USA
C. E. RUBIN, Seattle, Wash., USA
H. H. WANDALL, Copenhagen, Denmark

All communications pertaining to the publications in ACTA CYTOLOGICA should be directed to the Editorial Office, 5841 Maryland Avenue, Chicago 37, Illinois, U.S.A.

Requests for subscriptions should be sent to J. B. Lippincott Co., Subscription Manager, East Washington Square, Philadelphia 5, Pennsylvania, U.S.A. Volumes I, II, and III are still available. There will be a change in the format of the Journal and the frequency of its publication (six times per annum) with the following Volume V, scheduled to begin in January, 1961.

# TABLE OF CONTENTS


EDITORIAL . . . . . iv

LETTERS TO THE EDITORS . . . . . 167

REVIEW ARTICLE: *A Critical Review and Evaluation of Cytodiagnosis in Chest Diseases* by Heinz Grunze . . . . . 175

ORIGINAL ARTICLE: *Comparative Studies on the Degree of Proliferation of the Vaginal and Ectocervical Epithelium in the Hormonal Evaluation of a Patient by Means of Exfoliative Cytology* by Hans-Jürgen Soost . . . . . 199

THE SYMPOSIA BY CORRESPONDENCE: INTRODUCTORY REMARKS . . . 211

PROGRAM OF SYMPOSIA OF VOLUME IV, NOS. 2 & 3, 1960 . . . . . 213

SYMPOSIUM A: *Advantages of Various Techniques of Obtaining Material for Examinations* . . . . . 221

SYMPOSIUM B: *Endocervical Adenocarcinoma* . . . . . 271

SYMPOSIUM C: *Training of the Cytotechnologist* . . . . . 347

ALPHABETICAL LIST OF PARTICIPANTS OF PRESENT SYMPOSIA BY CORRESPONDENCE . . . . . 395

SYMPOSIA UNDER CONSIDERATION . . . . . 399

SPONSORING SOCIETIES . . . . . v

BUSINESS MATTERS OF THE INTERNATIONAL ACADEMY . . . . . x

# EDITORIAL

Recently, three additional national societies of cytology have joined ACTA CYTOLOGICA. It is a privilege to welcome the INTER-SOCIETY CYTOLOGY COUNCIL (future name: THE AMERICAN SOCIETY OF CYTOLOGY), the Italian Cytology Society: SOCIETÀ ITALIANA DI CITOLOGIA CLINICA E SOCIALE, and the German Cytology Society: DIE DEUTSCHE GESELLSCHAFT FÜR ANGEWANDTE ZYTOLOGIE.

In addition to THE INTERNATIONAL ACADEMY OF GYNECOLOGICAL CYTOLOGY, the Brazilian Society of Cytology: SOCIEDADE BRASILEIRA DE CITOLOGIA, and the Mexican Cytology Society: ASOCIACION MEXICANA DE CITOLOGIA EXFOLIATIVA will continue to use ACTA CYTOLOGICA as their official periodical.

The Journal is now being used by almost all existing national societies of exfoliative or clinical cytology, and it is hoped that it will serve as a central organ, providing readers with an international journal which will publish the newest advances in the field of cytology.

With this final issue of Volume IV, 1960, the Journal will undergo a major change in its form and publication schedule: ACTA CYTOLOGICA will appear six times per annum (January, March, May, July, September, November) as of 1961. The print of the Journal will be regular letter-press type instead of the previously used IBM-offset-type. The size of the Journal will be slightly reduced. ACTA CYTOLOGICA will be published by the J. B. Lippincott Company, Publishers, Philadelphia 5, Pennsylvania, U.S.A. The editorial policy and the types of papers published in the Journal will remain essentially unchanged. The new publisher has kindly consented to publish original articles in their original languages in addition to a complete English translation, if the manuscripts are submitted through their respective national cytology societies.

At this occasion acknowledgement seems due to our editorial office staff which has carried an extensive workload in starting the Journal, in providing the proof reading and translations of the manuscripts: Miss Catherine M. Keebler and Miss Margaret J. Keranen, and recently Mrs. Roberta G. Rautio, all of the Laboratory of Exfoliative Cytology of the University of Chicago, have voluntarily contributed their time and efforts in the accomplishment of editorial tasks.

The Journal was started with generous grants from The Cancer Research Foundation, and it could not have been maintained without this support. It is, therefore, a pleasure and a privilege to thank Mr. Maurice Goldblatt, Chairman of the Board, Dean Lowell T. Coggeshall, President, and the other officers of the Foundation for their generous support of the Journal.

GEORGE L. WIED

# LETTERS TO THE EDITORS

## SUGGESTION OF AN INTERNATIONAL INQUIRY ABOUT THE PROGRESS MADE DURING THE PAST DECADE IN THE EARLY DIAGNOSIS OF CERVICAL CARCINOMA BY CYTOLOGICAL METHODS

TO THE EDITORS:

A decade has elapsed since the International Congress of Obstetrics and Gynecology at New York City on May 14-19, 1950, where the Stage O or carcinoma in situ of the cervix was acknowledged. From that time the cytologic studies by the vaginal smear method have been performed all over the world and by this method an increased number of early cervical carcinomas has been discovered.

In 1948 a statistical evaluation of the World Health Organization made known that among 100 women affected by carcinoma of the cervix, who came to consult clinical hospitals or cancer institutes, 12 were operable, 33 were operable with few probabilities of success and 55 were absolutely inoperable. Also from the statistics of Heiman, Kottmeier and Sagerdhal published in 1953 (concerning previous years), it appears that in 64,236 cases, at the moment of diagnosis, the cervical carcinoma was Stage I in 16.5%, at the Stage II in 36.1%, at the Stage III in 35.7%, at the Stage IV in 11.7%.

Knowledge of the progress that has been made during the past decade for recognizing cervical cancer in its incipiency is still short and fragmentary, because the material is not homogeneous and the periods, to which the data refer, are not always the same. It would be, therefore, very advisable that an international inquiry be made in all the clinical hospitals or cancer institutes and in every hospital with a well-equipped cytology center.

This inquiry should be conducted with uniformity of principles and should refer to the years from 1951 to 1960; all the cases of cervical carcinoma diagnosed in Stage O and I should be reported every year. It also would be advisable to know if the cytological examinations are associated with colposcopic, colpomicroscopic, phase-microscopic and electron-microscopic investigations.

From scientific evaluation of accumulated material and from accurate comparison of the results, we would be able to evince the progress really obtained in the early diagnosis of cervical carcinoma and to draw useful indications as to what should be done in the future.

The inquiry also seems suitable in view of recent unfavorable judgments expressed in the systematic detection method by means of the vaginal smear examination. Indeed Redon and Lavour have affirmed that the use of this method is very expensive and the efficiency is low; they have quoted the report of Fernandez Ruiz at the IV Congress of Union Professionnelle Internationale de Gynecologues et Obstetriciens (U.P.I.G.O.) held in Lisboa on September 1959, from which results only 0.7% of 2,228,000 cases collected were positive.

In the Department of Obstetrics and Gynecology of the University of Pavia, where the employed diagnostic investigations are the cytological examination of vaginal and endocervical smears, of cervical swab smears, biopsy directed by colposcope and, in positive or suspicious cases, the extensive biopsies, I did research on the above matter until 1958. From the statistics collected the results show that the percentage of the cases diagnosed at the Stages O and I has increased from 6.6 in 1950 to 21.0 in 1958.

Bibliography

Heyman, J., Kottmeier, H. L., and Segerdahl, C. O.: Acta Obst. Gynec. Scand. 32:65, 1953.

Moracci, E.: Scritti Ost. Gin. 12:212, 1958-59.

Redon, H. & Lacour, J.: Enc. Mod. Chir., Gynécologie, 597 C 50, Ed. techniques, Paris, 1960.

Prof. Espedito Moracci, M.D.
Director, Department of
Obstetrics and Gynecology
University of Pavia
Pavia, Italy

# SHOULD A LESION BE TREATED WITH SURGERY AFTER IT HAS BEEN SHOWN CYTOLOGICALLY THAT THERE IS LITTLE OR NO RADIATION RESPONSE PRESENT? IF SO, WHEN WOULD SURGERY BE PERFORMED?

## TO THE EDITORS:

### CYTOPROGNOSTIC STUDY

Considering the difficulties involved, the clinician needs every available method to evaluate the progress of a carcinoma of the cervix undergoing radium therapy. The vaginal cytological smear seems to yield useful information in this matter.

It seems that patients whose tumors have been already treated with radium at the time of hysterectomy show a much longer survival rate than those whose pathological specimens showed active neoplastic cells. Most cervical carcinomas are radiosensitive. However, they seem to respond with varying rapidity to radiation in a way that is not clear to us at the present time. It seems that there is a "time factor," the time necessary to "sterilize" a tumor, which apparently does not depend on the clinical stage of the tumor at the beginning of the treatment or on the histological type or on the applied radium dosage. This time period, arbitrarily set by surgeons, can range from three to six weeks up to three months. This period seems to us insufficient in certain cases where the tumor is not completely inactivated and yet too long a period in other cases where the patient feels well and finally even refuses surgery.

Moreover, in most cases the pathologist who receives a specimen from an irradiated total hysterectomy ignores the history of the patient. In addition, the specimens show various stages of development, i.e., the dosages and applications of radium have been different as well as the time which elapsed between the insertion of radium and the hysterectomy. Therefore, it becomes very difficult for him to draw valid statistical conclusions.

This is why we thought it would be very interesting to compare the progression of the disease in cases of irradiated tumors. Fortunately, we were able to assemble a number of cases which had been submitted to the same scheme of treatment. These cases had been followed up with vaginal smears every six months, some of them for more than ten years. We wished to investigate whether or not the vaginal smear was valuable as a method of prognosis. It soon became evident that only those patients who had undergone the same type of treatment could be classified in the same series (from the general as well as from the cyto-prognostic standpoint). Variations of dosages and mode of radium application, surgical techniques, the interpretation by the cytologist and the pathologist represent a possible cause for statistical error.

Although we have examined hundreds of smears from 150 patients undergoing irradiation, we can present only a very small number of cases here because only a few patients with carcinoma of the uterine cervix at the same clinical stage received the same radiation therapy, had the same radiologist and had repeat smears examined by the same cytologist. We did not take into account the histological differences in the initial biopsies (before surgery) because the surgical specimens always reveal a marked structural polymorphism of the carcinoma of the cervix and at different sites of the sections. Because of these required conditions we were only able to present complete studies on 43 patients out of a total of 150 regularly followed cases.

In Table I we immediately see that out of 49 cases, 11 smears were uninterpretable. This abnormally high percentage of non-interpretable smears only occurs in irradiated patients. Owing to this fact it is not possible in vaginal cytology, as a general rule, to obtain a smear prior to 48 hours after vaginal douche. Moreover, certain patients who have completed radiation therapy continue to have a vaginal discharge for several weeks. In spite of repeated smears we have not been able to obtain one interpretable preoperative vaginal smear.

1. Among the 18 negative preoperative smears, 13 were confirmed histologically as negative and, therefore, could be considered as valuable. Among the latter 13 patients, 10 survived between 5 and 9 years and 3 died: one definitely of carcinoma and the other two (65 and 71 years of age, respectively), a final diagnosis of a neoplastic disease could not be made. Thus, taking into account the 3 patients who died, there still remains a five year survival rate of 72%.

2. Fifteen preoperative vaginal smears had been found positive. In these cases no consideration was given to cytology and the patients were operated upon, thus making possible examinations of the uterus and its surrounding tissues, which represents the best way of checking the method. Among these 15 cases, 5 were confirmed by histological examination, and 2 of the 5 patients died in less than 5 years; 2 are still living and have had positive smears and clinical signs of metastases. In only one case, in spite of positive cytology confirmed by histology, the operation seemed to have cured the disease, since the patient is well and has had negative smears for more than 8 years.

On the other hand, 7 out of the 15 positive smears have not been confirmed by histology, which represents a priori an impressive percentage of 50 false results. Undoubtedly, we know that it is difficult to differentiate between the irradiated, inactivated cells and the irradiated, potentially malignant cell; this difficulty may express most of our errors in the beginning of our use of cytology. We also must appeal to the other favorable side of cytology, to the chemical progression of the disease without considering only the histological prognosis. Thus it seems that in the 7 cases which displayed positive cytology with negative histology 3 have died within less than 5 years and the remaining 4 are alive up to the present time (a period of 4 years). Finally, among the 14 positive smears one had suspicious histology and is still alive after 2 years.

In this series of positive smears all patients have died or are in poor condition except four. Only one out of these latter cases has passed the crucial five year period. Thus, it is at the present not possible to give a global five year survival rate.

On the one hand, where patients had a negative smear, the survival rate after surgery is 72% at the 5 year period. On the other hand, where patients had positive or suspicious smears on the follow-up, the period was not long enough to give a complete five year statistical evaluation. Only a 2 year evaluation with 50% survival rate could be noted. It is only logical to believe that this figure will be even lower after the crucial five year period.

3. Five preoperative smears have been classified as suspicious. Two have positive histology and one

Table I

Survival Rate Correlated with the Results of Vaginal Smears of Patients Treated with Radium or Radium Plus Surgery

| | |
|---|---|
| Total of 59 patients treated with radium | |
| 43 patients treated with radium plus surgery<br>6 patients treated only with radium<br>49<br>11 of the total number of patients proved to have uninterpretable smears<br>60 | |
| 18 NEGATIVE SMEARS | |
| 13 confirmed by histology as positive | 10 survived up to 9 years<br>2 died from other causes than cancer (65 and 71 years of age)<br>1 died from cancer |
| 3 negative histologically | 2<br>1 true false positive |
| 2 cases have not had surgery and have survived up to 5 years | |
| 15 POSITIVE SMEARS | |
| 5 confirmed by histology as positive | 2 survived 5 years<br>2 survived 2 years with positive smears<br>1 survived 8 years with negative smears |
| 7 negative histological | 3 died within less than 5 years<br>4 survived 3 months to 4 years |
| 1 suspicious histological; survived 2 years after radiation<br>2 cases who did not have surgery; both died | |
| 4 SUSPICIOUS SMEARS | |
| 2 confirmed by histology as positive | 1 survived 8 years<br>1 survived 2 years with suspicious smear |
| 1 reported by histology as suspicious | Patient still living, but ill |
| 1 patient inoperable | |

had a suspicious histological finding. This would mean that suspicious vaginal smears correspond to real neoplastic lesions and that moreover, our criteria of malignancy should not have been used too liberally.

The general impression which may be derived from this statistic, although moderate, seems to be this: On one hand, patients operated upon who have had a negative smear have at least a five year survival rate of 72%. On the other hand, those patients operated upon with a positive or suspicious smear showed a two year survival rate of about 50%. The follow-up was too short a period to compile a complete five year statistical evaluation, but we have only 50% survival after two years, and that would mean that the percentage at the five year period would be still lower.

In other words, the percentage of survival after surgery is undebatably much larger in the group of patients with negative smears. For these reasons we have asked ourselves whether or not it would be preferable to suggest to the surgeon, within limits that would not violate clinical objection, to wait until the smear becomes negative, either by several radium applications or evey by discontinuation of any therapy.

With good reason we have been told that if the tumor, after a certain time after irradiation therapy, still discloses cytological and histological signs of malignancy, one should be entitled to conclude that one deals with a true radiation resistant tumor. Therefore, the prolongation of radiation therapy or the simple discontinuation of all therapy, hoping that a delayed therapeutic effect will arise, plainly represents an error and for the patient an irreversible loss of time.

Case #1: The smear or Mrs. H. exhibited a Class V on February 17th, 1956, the biopsy confirmed that we were dealing with an undifferentiated epidermoid carcinoma (epithelioma). The smears during therapy were practically identical with the initial one. The results were as follows:

One month after radium application: Class IV with no appreciable signs of radiation effect. Three months after radium application: Class IV, with no appreciable sign of radiation effect.

The surgical specimen after total hysterectomy showed perfectly viable cords of the epithelioma and complete lack of any radiation changes, histologically.

Except for this patient, who has been doing well for three years, our attitude has been justified by the fact that almost all the smears eventually become negative sooner or later.

Far from being able to predict the precise date

Table II

Classifications

| CLASS | CLASSIFICATION OF PAPANICOLAOU BEFORE TREATMENT | OUR CLASSIFICATION AFTER TREATMENT |
|---|---|---|
| I | No abnormal or atypical cells found. | Normal smear. |
| II | Atypical cells found, but without structural abnormalities. | Persistence of irradiation pattern. |
| III | Abnormal suspicious cells found, but not pathognomonic for malignancy. | Presence of irradiation patter and presence of suspicious cells for malignancy. |
| IV | Moderate number of malignant cells found. | Moderate number of malignant cells found, with or without persistence of irradiation changes. |
| V | Numerous cells found conclusive for malignancy. | Numerous malignant cells found with or without persistence of irradiation changes. |

of when the smear will become negative, we limit ourselves to determining the first appearance of radiation changes. We plot the results of the smears, systematically taken during the 15 days post radium insertion, on a single graph; thus making it immediately evident for the gynecologist to note the time when the first changes occur.

Case #2: Less than one month after radium insertion, Miss N. exhibited a negative smear. For extramedical reasons she was operated on at a later date. She remained negative both cytologically and histologically. For 9 years she has been doing fine from both a clinical and cytological standpoint.

Case #3: Three months after radium insertion, Miss S. (62 years of age) was operated, i.e., the same time as the Case #2. However, Miss S. exhibited a positive preoperative smear. She also presented a cytological curve that turned negative at the time of the surgical interference and again became positive several months later. Simultaneously a neoplastic lump appeared at the site of the vaginal operative scar. Subsequent radiation therapy again brought about a remission—much shorter than the previous one. The patient died 2-1/2 years after commencement of therapy with recurrence of positive smears.

These two cases and all the others which are analogous show that the clinical course is undoubtedly more favorable when the interference achieved the extirpation of a definitely or momentaneously quiet tumor rather than a carcinoma in progressive evolution. It also exhibits that vaginal smears permit a valid appreciation of the local state.

The observation points seem to be reduced to a minimum at the present time:

1. The eruption of lymph node metastases never appears early enough to represent a cause for false negative vaginal smears at the time when the indication for active interference has come.
2. All cytologists are sufficiently aware of the pitfalls present in inflammatory smears and they are careful not to give a cytological evaluation before therapy has been initiated.

## CONCLUSIONS

For practical purposes, we have adopted the following routine:

1. To follow the patient after radium therapy with repeated cytological smears, not excluding clinical examinations, and to wait several subsequent negative smears (at least two).
2. To perform the appropriate surgical procedure in a "negative state."
3. To restrain ourselves only to radiation therapy in cases of persistently positive smears and perhaps according to circumstances may be followed by a second radium application.

We believe that by applying this method we were able to improve the overall survival rate of our patients.

Colette Marsan, M.D.
Laboratoire de Cytologie
de la Clinique
Chirurgicale de L'Hopital Beauton
Paris, France

# DO SPECIFIC MORPHOLOGICAL ALTERATIONS OF A SQUAMOUS EPITHELIAL CELL EXIST AFTER RADIATION?

## TO THE EDITORS:

Reference is made to the discussion between A. A. Arrighi and L. V. Bertalanffy and F. D. Bertalanffy in the paper "Morphology of the Irradiated Squamous Epithelial Cells (RR cells)" by A. A. Arrighi, published in Acta Cytologica, Vol. III, No. 2, 1959, p. 352, concerning the question if the morphologic alterations observed in irradiated squamous epithelial cells have to be considered as "unspecific degenerative processes" (L. and F. Bertalanffy) or if they might represent "specific, although not constant, modifications produced by the actinic therapeutic agent" (A. A. Arrighi).

In attempts of a local cytostatic treatment of carcinoma in situ of the cervix uteri, we found changes of the normal and atypical squamous epithelial cell after prolonged and continuing local use of "endoxam,"* a cytostatic drug, which are morphologically like the ones caused by radiation. These changes are shown in the accompanying figure.

Figures 1-3 show the increase in size of both nucleus and cell in all layers under endoxan-therapy with an undisturbed nuclear-cytoplasmic ratio. Figures 2 and 4-6 show small and large vacuoles in the cytoplasm of parabasal and intermediate cells under endoxan-therapy. The increase in size of nucleus and cell can be seen also in these pictures as well as the immigration of leucocytes into the damaged cell. The alterations of the nuclei under endoxan correspond with the radiation changes of nuclei; karyolysis (Fig. 1, 8), karyorrhexis (Fig. 9) wrinkling of the nuclear surface (Fig. 7, 8) and the presence of multiple nuclei are observed under cytostatic therapy.

The sequence of manifestation of the morphological changes under cytostatic therapy seems to be important also: In daily cytostatic controls we observed at first changes of the cytoplasm to be followed within a short period of time, by the changes of the nuclei described above. Similar relations can be found in the squamous epithelial cells after radiation therapy.

From the above findings we conclude that alterations of nucleus and cytoplasm are not specific for a certain agent but that the squamous epithelial cell respond to different kinds of damaging agents (cytostatica, radiation) with the same alterations of nucleus and cytoplasm, i.e., these changes are of an unspecific nature in the meaning of degenerative processes in nucleus and cytoplasm (L. and F. Bertalanffy).

Bibliography

1. Hillemanns, H. G. and Wagner D. Arch. Gyn. 192: 277, 1960.

Fig. 1.

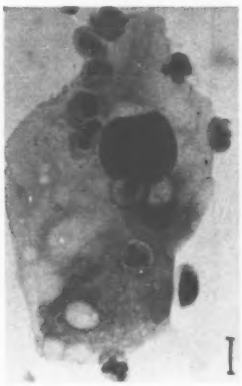


Fig. 2.

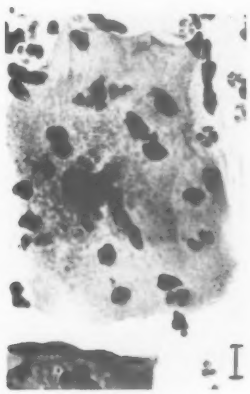


Fig. 3.

Fig. 1. Increase in size of nucleus and cytoplasm of a parabasal cell. Case W/905/58/5/St. 3; Papanicolaou stain, 840x.

Fig. 2. Increase in size of nucleus and cytoplasm of an intermediate cell. Beginning vacuolization of the cytoplasm and immigration of leukocytes in the cytoplasm of the damaged cell. Case M/b/III/St. 3; Papanicolaou stain, 520x.

Fig. 3. Increase in size of nucleus and cytoplasm of a superficial cell. Case x2134/60/St. 5; Papanicolaou stain, 430x.

*"endoxan" = phosphorylated N-Oxyd-Lost= (B 518), Asta-Werke, Brackwede, Westfalen, Germany.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 4. Vacuoles in the cytoplasm of a damaged basal cell; increase in size of the nucleus and cell. Case x2383/60/St. 3; Papanicolaou stain, 780x.

Fig. 5. Vacuoles in the cytoplasm of a damaged basal cell; increase in size of the nucleus and cell. Case x2383/60/St. 5; Papanicolaou stain, 1200x.

Fig. 6. Vacuoles in the cytoplasm of a damaged parabasal cell; increase in size of the nucleus and cell; immigration of leukocytes. Case M/c/II/St. 1; Papanicolaou stain, 820x.

Fig. 7.

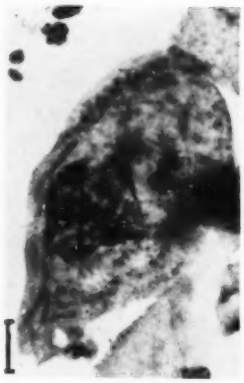


Fig. 8.

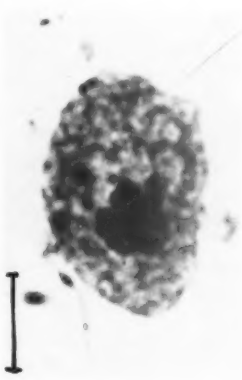


Fig. 9.

Fig. 7. Nuclear alteration of the damaged cell: multiple nuclei; wrinkling of the nucleus. Increase in size of the nucleus and cell; beginning vacuolization of the cytoplasm. Case W/905/58/St. 1; Papanicolaou stain, 740x.

Fig. 8. Nuclear alteration of the damaged cell: beginning karyolysis; wrinkling of the nucleus. Increase in size of the nucleus and cell; beginning vacuolization of the cytoplasm. Case M/c/III/St. 1; Papanicolaou stain, 750x.

Fig. 9. Nuclear alteration of the damaged cell: karyorrhexis. Case x2383/60/St. 46; Papanicolaou stain, 1500x.

Dr. D. Wagner
Univ.-Frauenklinik
Freiburg/Br., Germany

# CARCINOMA IN SITU—CYTOLOGICAL AND HISTOLOGICAL INTERPRETATIONS

## TO THE EDITORS:

Reference is made to Volume III, Number 1 in which there were conflicting interpretations of the lesions with the cytologic or histologic findings of carcinoma in situ occurring during pregnancy which often subsequently regresses after the termination of pregnancy. There are some who will not identify these lesions as carcinoma in situ because of their spontaneous regression and more or less imply that spontaneous regression of itself excludes the possibility of malignancy. Concomitant with this there is, of course, the exfoliation of cells which meet the cytologic criteria of malignancy and subsequently with the regression of the histologic lesion there is a disappearance of malignant cells from the exfoliated cells.

It becomes apparent that if the histologic criteria for the diagnosis of carcinoma in situ are rejected merely because the lesion, which would otherwise be considered malignant, regresses there will develop a confusion of semantics that could serve as a great handicap in classifying lesions, evaluation of treatment, interpretation of cytology and in determining the natural history of lesions.

It would seem to be more reasonable to interpret histologic and cytologic findings strictly by morphologic criteria and not have the morphologic diagnosis altered by the occurrence of spontaneous regression. This will allow us to study groups of apparent carcinomas in situ which spontaneously regress. If such a lesion is not classified as carcinoma in situ because it did spontaneously regress then it will be diagnosed as a benign lesion and this could be in error. It seems very possible that a cell could be inherently malignant and the behavior of this cell could depend upon host factors as well as the malignant propensities that reside in the cell. The host factors may be factors that at one time may allow the inherently malignant cell to proliferate, such as during pregnancy, and at another time the host factors may oppose the proliferation of the inherently malignant cell such as in the postpartum or nonpregnant state.

It has been recently shown that patients with malignant disease can be inoculated with malignant tissue and this tissue will frequently grow whereas patients without malignant disease are not a fertile soil and fail to take transplants of malignant tissue. Apparently patients with malignant disease have lost an immunity to malignant tissue and the normal person has an immunity against malignant tissue.

If the logic of those who say that a carcinoma in situ is not a carcinoma in situ because it regressed is applied to the identification of a pathogenic bacterium it would follow that a bacterium could not be considered a pathogen unless it was actually causing an infection. Of course, we don't identify a pathogenic organism by this criteria. A pneumococcus is a pathogen regardless of whether or not it is causing a pneumonia, etc. A bacterium is a pathogen because it has the inherent capacity to cause an infection and not because it always causes an infection. Perhaps a malignant cell is malignant because it has the capacity of unrestrained and sustained growth but it need not always behave this way because the host may have factors that oppose this malignant behavor of a cell. It does not mean the malignant behavior or capacity is not present in the cell.

The writer does not wish to give the impression that the morphology of a cell determines the benign or the malignant nature of a cell. The nature of a cell is solely determined by its behavior. If a cell behaves in a malignant manner then the morphologic findings of this cell which serve to identify it, also serve to classify it as malignant regardless of whether or not the nuclear features conform to the generally accepted features of malignancy. Conversely, if a cell has malignant features but fails to behave in a malignant manner it cannot be considered malignant but it is imperative that one keep in mind a possible exception to this statement and this is that a cell with a malignant nature may be opposed by immune factors in one host that restrain its growth or actually destroy it and that this same cell in another host may enjoy unrestrained and invasive growth.

It would seem that this is a statement of fact as demonstrated by the carcinomas in situ that spontaneously regress. To assume that the lesions that regress are benign because they regress would seem to be a presumption and that logic dictates that the burden of proof rests with those who claim that these regressible lesions are inherently benign.

In the future it may be determined that there are cells which have the nuclear features of malignancy and these cells are inherently malignant and that there are other cells morphologically indistinguishable from these inherently malignant cells but they are inherently benign. If this undesirable day comes to pass it would have to be acknowledged by cyto-pathologists that cytology cannot reliably detect carcinomas during pregnancy. The possibility of this situation cannot be appraised unless the cells which possess the morphologic features of malignancy are called malignant regardless of the regression of the lesion. In time, large series of these cases will be collected and it may be possible to retrieve viable cells of the type seen in carcinomas in situ during pregnancy and successfully start tissue cultures where more intimate studies could answer the above stated problems.

The thoughts contained in the foregoing may be obvious to everyone concerned and tacitly acknowledged by cytologists. However, they weren't explicit in the symposium and it would seem to be a logical stand to take.

Francis X. MacAulay, M.D.
Director of Laboratories
Orangeburg Regional Hospital
Orangeburg, South Carolina, U.S.A.

A SPECIAL SYMPOSIUM BY CORRESPONDENCE ON CARCINOMA IN SITU HAS BEEN FINISHED RECENTLY AND WILL BE PUBLISHED WITHIN THE NEAR FUTURE. IN ADDITION TO THIS A SPECIAL PANEL ON HISTOLOGICAL DEFINITIONS HAS BEGUN ITS WORK UNDER THE CHAIRMANSHIP OF DR. JAMES W. REAGAN, PROFESSOR OF PATHOLOGY, WESTERN RESERVE UNIVERSITY, CLEVELAND, OHIO. OUTSTANDING SPECIALISTS ARE MEMBERS OF THE PANEL. THIS PANEL IS WORKING CURRENTLY BY CORRESPONDENCE TO LAY THE FOUNDATION FOR DELIBERATIONS IN PERSON AT THE TIME OF THE FIRST INTERNATIONAL CONGRESS OF EXFOLIATIVE CYTOLOGY IN VIENNA, AUSTRIA (AUGUST 31 TO SEPTEMBER 2, 1961).—Ed.

ACTA CYTOLOGICA · 1960

Vol. IV REVIEW ARTICLE Nos. 2 & 3

# A Critical Review and Evaluation of Cytodiagnosis in Chest Diseases

HEINZ GRUNZE

## I. INTRODUCTION

A review of cytodiagnosis in diseases of the thorax might be considered from the viewpoint of the clinical pathologist or from that of the clinician. The former is concerned primarily with the relative merits of various methods; the latter with the overall usefulness of cytodiagnosis in a given case.

In this study, both aspects of cytodiagnosis will be considered, but the main emphasis will be toward the viewpoint of the clinician who is confronted with a diagnostic problem either discovered on the chest film or during the physical examination. Sometimes the clinician's problem begins with determining the origin of the lesion, since, not infrequently, it is difficult to establish whether or not an abnormality arises from the pleura, the lung parenchyma or the mediastinum. In some of these cases the answer may be obtained by cytological studies. This localization of the lesion may be as valuable for the clinician as the decision of whether or not the lesion is malignant, although the latter is the cytologist's main concern.

Furthermore, the clinician appreciates any information concerning the histologic type, although he knows that this information is less precise than that obtained from histologic sections. However, correlated to the rest of the clinical findings, even this less precise information might have more diagnostic value to the clinician than the differentiation between a Class IV and V reading (according to Papanicolaou), because it might serve, in difficult cases, as a basis for further examinations (as in the search for the primary tumor) or in establishing the prognosis. This clinical attitude does not deny that such a classification is of significance to the clinical pathologist in evaluating his results.

In short, the chest physician dealing with individual cases generally prefers an interpretation oriented to his specific problems rather than a classification. More detailed description of non-malignant patterns might be helpful in this regard in the future.

Since the thorough and extensive studies of the late Dr. Chandler N. Foot, correlating histological and cytological findings (67–70), much progress has been made in connecting both the clinician's and the pathologist's points of view. This trend of clinical cytology seems to promise better results, especially in the field of Internal Medicine. As will be shown later, the problem of correlating the clinician's and pathologist's points of view was formerly not so common, because the clinician himself often performed the cytological examination.

From the Laboratory of Exfoliative Cytology, University of Chicago, Chicago 37, Illinois. Supported by a grant from the Illinois Division of the American Cancer Society and a Fulbright Fellowship. Present address: Privat-Dozent Dr. med. Heinz Grunze, II. Medizinische Universitaets-Klinik der Freien Universitaet Berlin (Direktor: Prof. Dr. Heinrich Bartelheimer) Spandauer Damm 130, Berlin-Charlottenburg 9, Germany.

## II. HISTORICAL DEVELOPMENT OF CLINICAL CYTOLOGY IN CHEST DISEASES

This historical survey commences with the first papers available to this reviewer on cytology in the 19th century and ends at the time when Papanicolaou and Traut published their monograph, *The Diagnosis of Uterine Cancer by the Vaginal Smear*, in 1943 (190). Although dealing with gynecological cytology, their encouraging results in this field of medicine resulted in a renaissance of all aspects of clinical cytology, including that of chest diseases.

The papers published after 1943 will be mentioned later in a section dedicated to the present status of cytodiagnosis in chest diseases.

A concept of the cell which was applicable in biology as well as in medicine was developed at the beginning of the 19th century. It is surprising to note the rapidity with which the theories of Schleiden (238, 239) and Schwann (240) were disseminated throughout Europe despite the technical difficulties at that time for exchange of scientific ideas and theories. Schleiden (238, 239) for plants and Schwann (240) for animals established the general concept of the cell as the basic unit in living and growing organisms. The earlier attempts of Malpighi, Corti, Brown, Hooke, and others to define the elementary life units are reported in detail by Aschoff (4) and co-workers and described more recently by Rabe (213) and by Hughes (120). The latter, a monograph on the *History of Cytology*, contains a comprehensive bibliography.

Many years before Virchow (266, 267) published his important general concept of cellular pathology, the patterns of isolated cells from tissues, fluids, and secretions had been studied in health and disease by several authors. Interestingly, more attention was paid to malignant diseases than to inflammatory changes. Figure 1 shows a picture from a book of Johannes Mueller* entitled *On the Nature and Structural Characteristics of Cancer and Those Morbid Growths Which May be Confounded With It*, published in 1838 in Berlin (183). This book contains microscopic criteria for the fundamental differentiation of malignant and benign tumors and, furthermore, groups these tumors into those with abundant connective tissue (sarcoma) and those with scant connective tissue (carcinoma). Although the author had some difficulties in interpreting the different microscopic patterns of cells, in

* J. Mueller quotes the cooperation of foreign colleagues and the merits of former investigators (Abernethy, Burns, Hey, Laennec, Valentin, Cruveilhier, A. Cooper, Hodgkin and others).

the text one can find elucidating statements which are still true today. For instance, the difference in the tendency for

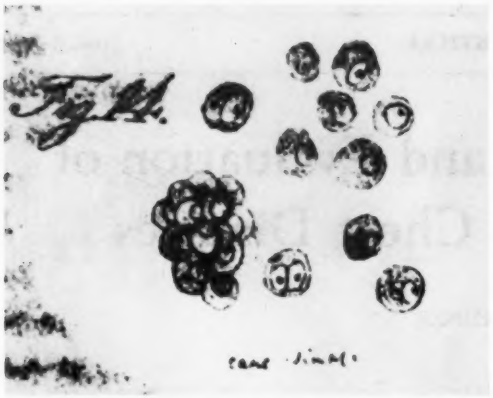


FIG. 1. From Mueller (1838) Original legend: "Cells with germinal cells and nuclei; from an extremely hard carcinoma mammae simplex which had passed into the ulcerated stage. Diameter of the cells 0.00048 to 0.00128 of an English inch." Reviewer's remark: Enlarged nucleoli and nuclear crowding are apparent.

cell exfoliation in carcinoma and sarcoma or benign lesions is pointed out by the following quotation: "In other cases, where microscopic cells still form the basis of the growth, these cells do not cohere, though in close apposition; but on the contrary, are free, may be separated from each other, and, when looked at under the microscope present the appearance of globules." In emphasizing the importance of these elements, he says: ". . . these minute globular cells, which form the real seminium morbi in several forms of carcinoma. . . ." Finally, referring to Schwann's cell theory, he adds: "Thus, then, as might have been anticipated, did the examination of morbid structure confirm Schwann's observations touching on the development and growth of healthy tissues."

This basic monograph was cited in most of the publications dealing with cytology in the 19th century. At that time it was typical that the microscopic examination of specimens included evaluation by both cytological and histological criteria. The few illustrations, which were expensive to publish at that time, contained approximately equal numbers of figures of cells and of tissue sections or squeezed tissue preparations. Even in the late 19th century this was true, as documented in Figure 9.

Other contributions came from France: Lebert (152), in 1851, wrote in his book, *Traité des maladies cancéreuses et des affections curables confondues avec le cancer*, a chapter entitled: "Élement spécifique du cancer: cellule cancéreuse." Describing the features of cancer cells, he notes their varying size, polymorphism, enlarged nuclei and nucleoli. Also, Lebert (152) seems to have inaugurated cytodiagnosis by needle puncture, a precursor of lung puncture. He writes: ". . . et une ponction explorative pourra lever au besoin tous le doutes. Si l'on a affaire à une kyste purulent ou séreux, un filet de liquide sort par la canule; si l'on tombe dans du tissu cancéreux au contraire, la canule, au lieu d'être mobile, se trouve plus solidement engagée, et s'il sort quelque chose, c'est une pulpe molle que l'examen a l'oeil nu, et, au besoin, le microscope font reconnaître comme cancéreuse." (In translation: ". . . and an exploratory puncture might remove all doubts. Dealing with a purulent or serous cyst, a liquid jet will come out of the canula; on the contrary, when entering cancerous tissue, the needle is surrounded by more solid structures and is less mobile. If some material is obtained, it is a soft pulp. Examining this macroscopically, and if necessary, microscopically, it is found to be cancerous.")

Another French author, Broca (25), made the impressive statement: "Le véritable cancer ne pardonne jamais" (True cancer never gives parole).

The first special studies and illustrations of exfoliated cells of the respiratory tract are found in the general monographs of Donné (47) in Paris, 1845 and Walshe (268) in London, 1846. In addition to a drawing of typical squamous epithelia of the mouth, the latter contains a description of characteristic sputa in lung cancer patients: "The expectorated matter varies in quality. It may be simple mucus or catarrhal, or purulent or bloody. In the latter case, the blood seems mixed thoroughly with serum, mucus or mucopus; and the sputa, thus composed, are opaque and commonly free from viscidity, though they may be somewhat glairy. The color of such sputa varies: it may be that of red current jelly, of black currant jelly, or pink encephaloid (cancerous) matter has sometimes been expectorated; cases observed by Bayle, Davies and Cannstatt, furnish most distinct examples of the fact." However, Walshe (268) mentions no attempts to perform a microscopic examination of the "encephaloid matter."

Donné's (47) work* is remarkable in a different way. It describes the morphology of cells in a large variety of specimens, such as blood, urine, and mucus from the nose, bronchi, gastrointestinal tract, prostate, vagina (Trichomonas vaginalis included) and shows also the first photomicrographs in medicine, taken by "Daguerréotypie." Similar to Johannes Mueller (183), Donné (47) tried to make a chemical analysis of the material examined. This monograph contains no studies on malignant growth but gives the first thorough information on normal and inflammatory cytology of different fluids and secretions. Even milk, colostrum, and crystallized particles in mucus are documented in Donné's atlas (47). Three pictures taken from this atlas may illustrate his achievements (Figs. 2–4).

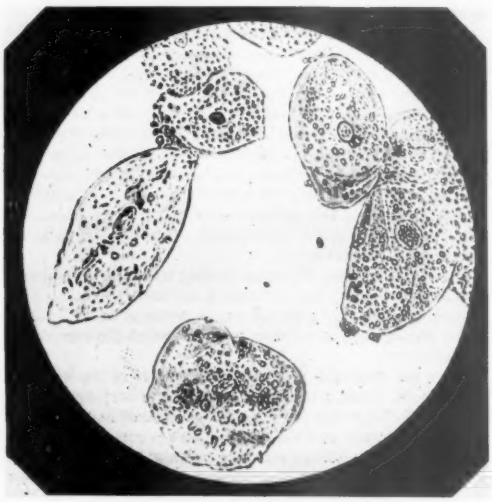


FIG. 2. From Donné (1845), Original legend: "Cellules épidermiques du mucus des membrances analogues à la peau, sécrétant un fluide acide, comme la sueur." (Squamous cells of mucous membranes similar to the skin, excreting an acid fluid-like sweat.)

* Reference to this monograph is made also in publications of Bollack (23) and Papanicolaou (194, 195).

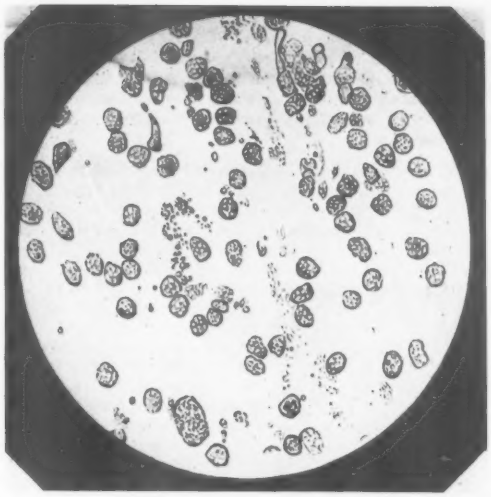


Fig. 3. From Donné (1845). Original Legend: "Globules muqueux du mucus proprement dit (mucus alcalin), tels qu'ils se présentent dans le mucus nasal et dans des bronches sécrété depuis un certain temps et ayant séjourné à la surface des membranes." (Mucous globules [leukocytes] of mucus [better defined as alkaline] as seen in mucus of the nose and the bronchi, secreted sometime before and having remained on the surface of the mucosa.)

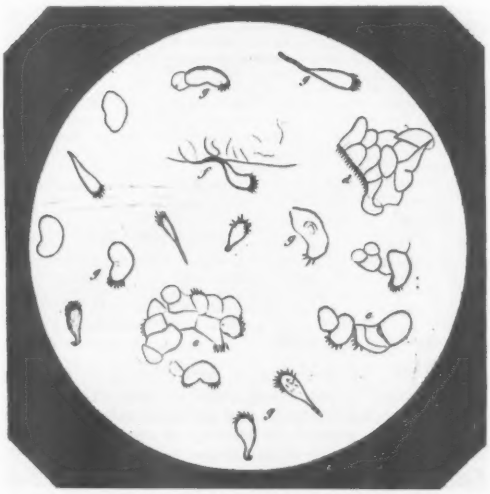


FIG. 4. From Donné (1845). Original legend: "Épithélium de la membrane muqueuse nasale d'un homme, arraché pendant la vie avec un polype. D, fragment de cette membrane avec les ciles vibratiles sur le bord. E, l'épithélium commensant a se désagréger. F, cellule d'épithélium pourvue de ses, cils vibratiles se separant et s'animant isolement. G, cellules animées nageant isolément dans l'eau ou elles sont plongées." (Epithelium of the nasal mucosa attached to a polyp which was pulled out during lifetime. D, fragment of this mucous membrane showing vibrating cilia at the edge of the cell. E, epithelium starting to separate. F, epithelial cell where cilia have been separated and more isolated. G, isolated cells moving in water.)

The first American author describing both the cytological features of normal and malignant tissues was Donaldson (46) in 1853. His studies were based on smears taken from the cut surface of the tissue. He described his technique as follows: "The mode we have employed has been simply to place between two pieces of glass a drop of the juice, obtained either from gentle pressure, or by scraping the cut surface with a scalpel, diluted with a little water. The cutting off of small slices with Valentin's knife, and examining the whole mass together, will exhibit, almost invariably more or less fibrous structure, but necessarily the lens employed must be much feebler, and the cell is not seen to the same advantage; moreover, the fibrous element is purely accidental, and is found in a vast number of tumors."

Another of Donaldson's (46) basic statements was concerned with the variety of cell forms found in the same tumor: "Some are disappointed on being told that the cancer element is not always of a uniform size, or even of a certain fixed shape; but they certainly do not doubt the existence of

Fig. 6.

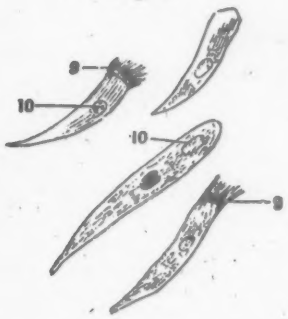



Fig. 8.

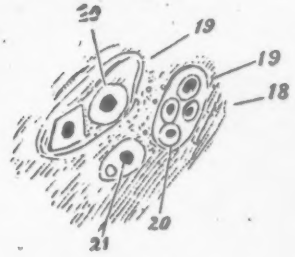



Fig. 9.

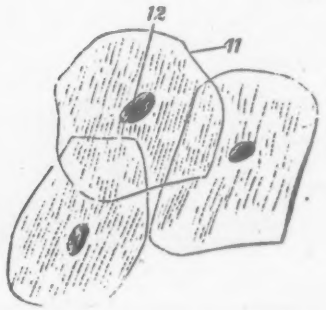



FIG. 5. From Donaldson (1853). Original legend:
"Fig. 6. Cylindrical and ciliated elements.
Fig. 8. Costal cartilage.
Fig. 9. Buccal epithelial scales."

epithelial cells because their shape and color is different on different surfaces. We will presently show that the great variety of form in the cancer element is a strikipg peculiarity characteristic of the disease. Notwithstanding their variable form, they all have points of unmistakable resemblance with each other. Out of one hundred individuals of a particular race, there may not be two with precisely the same features, yet about whom there could be no doubt as to their common origin. To use an expressive French phrase, they all have their 'cachet particulier.' "

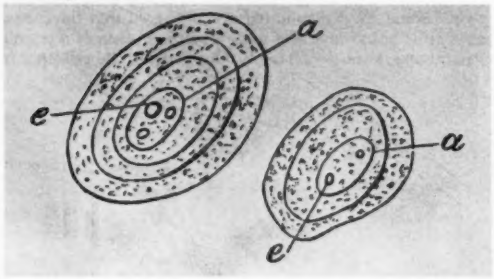



FIG. 6. From Donaldson (1853) Original legend: "Two concentric cancer cells; a—the cancer nucleus, the size of which is always in proportion to the innermost circle; e—the brilliant nucleolus." Describing these concentric cancer cells in the text of his paper, he adds ". . . sometimes a mass of epithelial cells are pressed together and present somewhat this appearance."

A few years later, in 1860, Beale (12) reported in his *Archives of Medicine* (London) the first cytological examination of spontaneously raised sputum in a case of cancer of the pharynx and adjacent tissue which was submitted by his colleague, Dr. Newman. Figure 7 shows the detailed illustration of this paper, demonstrating strikingly the patterns of a cornifying squamous cell cancer. The legend, which was added by the author in a later publication (1879) still shows the difficulties in interpreting the polymorphous details of this cancer type.

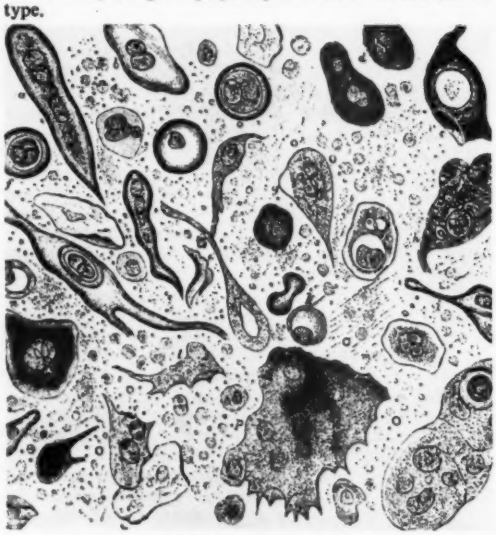


FIG. 7. From Beale (1860)

In spite of this encouraging report from Beale and in spite of repeated notes on macroscopic visible parts of tumor growth in sputum—cited by Walshe (268) and Wandall (269), some time passed before the first successful cytological examination, in a patient with lung cancer, was performed. It was Hampeln (98) who first published in the *Petersburger Medizinische Wochenschrift* in 1887 a case of primary carcinoma of the lung and pleura. Figure 8 shows a portion of this article, illustrated by a drawing of cancer cells and in which the author states that the cytological examination performed five months ante-mortem was useful in establishing the diagnosis.

des Auswurfs. Die c. 5 Monate vor dem Tode vorgenommene Untersuchung ergab in demselben ausser Detritus und kleinen, feinkörnigen Rundzellen, zahlreiche polymorphe, keulen-, spindelförmige etc. Zellen von auffallender Grösse mit körnigem Inhalt, mit einem oder mehreren Kernen, kürzere Zellengebilde, welche als »Krebselemente« des Sputums

bereits Beachtung gefunden haben. Das gleiche Bild erhielt man bei Untersuchung des in den Bronchien der erkrankten Lunge enthaltenen spärlichen Schleims, sowie des Geschwulstsaftes. Ueber diese verdanke ich Dr. K r a n n h a l s folgende Beschreibung:

»Ausser den grossen, mehr spindel- oder keulenförmigen Zellen, welche aus dem Geschwulst-Stroma zu stammen scheinen, dürften namentlich die Mundepithelien ähnlichen, rundlich-eckigen Zellen charakteristisch sein. Sie finden sich im *Bronchialschleim* und im *Geschwulstsaft* in gleicher Weise. Ihr Zellendurchmesser ist 0,002—0,025 Mm., ihr Kerndurchmesser 0,012—0,0125. Sie bilden auch den Inhalt der *Krebsalveolen*. Die spindel- und keulenförmigen Zellen finden sich reichlich im Geschwulstsaft, im Bronchialschleim spärlicher. Ihre Dicke ist meist 0,0125, an der dicksten Stelle bis 0,02, ihre Länge sehr verschieden; wo sie als Faserzellen auftreten bis zu 0,1 meist aber 0.05 Mm.«

FIG. 8. The translation of the text is as follows: "The cytological examination of the sputum, performed approximately five months before death, revealed, besides debris and fine granules containing small round-shaped cells, numerous polymorphic club-shaped and spindle-shaped cells of striking size with a granular-like content and single or several nuclei. Furthermore, there were shorter cells, already known as cancer elements of the sputum. The same picture was seen when examining both the scanty mucus in the bronchi from the ill lung and the juice of the tumor. For a description of both I have to give full credit to Dr. Krannhals: 'Besides the large club-shaped and spindle-shaped cells probably originating from the stroma of the tumor, cells with roundish edges similar to epithelia of the mouth seem to be characteristic. They are found in both the mucus of the bronchi and in the tumor juice. Their cellular diameter is 0.0002q0.025 mm and their nuclear diameter is 0.012q0.0125. These cells form also the content of the cancerous alveoli. Occurring often in the juice of the tumor, spindle-shaped and club-shaped cells are rarer in the mucus of the bronchi. Whereas their thickness is mostly 0.0125 mm (at the thickest spots up tp 0.02) their length variesl mostly 0.05 mm but goes up approximating to 0.1 mm when occurring as fiber cells.' "

It is not only this classical description of a cornifying spindle-shaped squamous cell cancer of the lung that makes this report so important but also the fact that both Hampeln and Krannhals proved the identity of the exfoliated cells found in sputum with those forming the malignant growth. Furthermore, Hampeln, in a paper published in 1897 (99), is to be credited with the first thorough description of normal and non-malignant cellular elements in sputum Figure 9 shows some of his illustrations.

In 1919 Hampeln (100) again first reported a series of cases utilizing sputum cytology for the diagnosis of bronchogenic carcinoma. Thirteen of twenty-five cases of carcinoma yielded positive cytology for malignancy. This report was the first attempt at an objective statistical evaluation of the procedure; prior to this only single cases had been published-these case reports have been reviewed by Wandall (269) and Grunze (88) in 1955. One of those reports, recording two cases, was that of Betschart (18) in Switzerland (1895). In order to document the previously-mentioned custom of this early period to illustrate both the histologic and cytologic patterns of a case, his drawings are reproduced here (Fig. 10). However, accord-

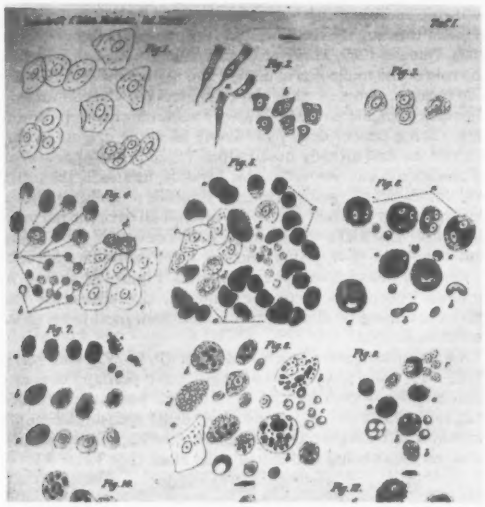


FIG. 9. From Hampeln (1897). Translation of the original legend:

1. Epithelia of the pharynxl epiglottis and chorda vocalis.
2. Cells of the tracheal and bronchial mucosa, superficial (a) and deeper (b) layer.
3. Normal alveolar cells.
4 and 5. Cells from the sputum of a strong smoker. Transparent smoke-grayish balls. (a) Smoke cells, brownish-grayish dark; (b) leukocytes; (c) pharynx-epithelia; (d) alveolar cells.
6. Cells from the transparent black sputum of a coal miner, stained with methyl-violet. This procedure brightens the cells so the nuclei and nucleoli bscome more prominent. Some cells show a bright violet nucleus. Many free nuclei of the 'coal cells.' (a) Coal cells; (b) free nuclei; (c) luekocytes.
7. Cells from the transparent morning sputum in a case of chronic smoker's catarrah. (a) "Myelin" cells and free "myelin" granula; (b) "myelin" cells brightened and homogenized by treatment with glycerine; (c) stained with methyl-violet.
8. Sputumn cells in a case of pulmonary infarction (mitral stenosis).

   Second day after infarction:

   (a) Epithelia of the pharynx.
   (b) Heart failure cells. Background bright white. Yellow and brown shiny pigment clods. The nuclei are covered. After treatment with methyl-violet the oval nuclei showing distinct nucleoli become more prominent. Sometimes 2 to 5 nuclei are in one cell; in other instances they are lying free.
   (c) Erythrocytes.
   (d) "Myelin" cells.
9. Five days after infarction: The large cells have disappeared. The rest are small bright yellowish cells.

ing to this reviewer, some alveolar cells might be misinterpreted in Betschart's legend.

A monograph of Besançon and de Jong (19) gave a critical evaluation of all papers published in France until 1912. Most of the investigations listed in this book were concerned with inflammatory and congestive disorders of the respiratory tract. This applies also to another comprehensive survey on the cytology of the respiratory system which was published in Germany by von Hoesslin (117) in 1926. Hoffmann's (118) and Sommerbrodt's (249) findings in congestive heart failure and Liebmann's (154, 155) investigations in tuberculosis are extensively quoted in this monograph which also contains, in addition, detailed pictures and descriptions on the gross findngs of sputa in various disorders.

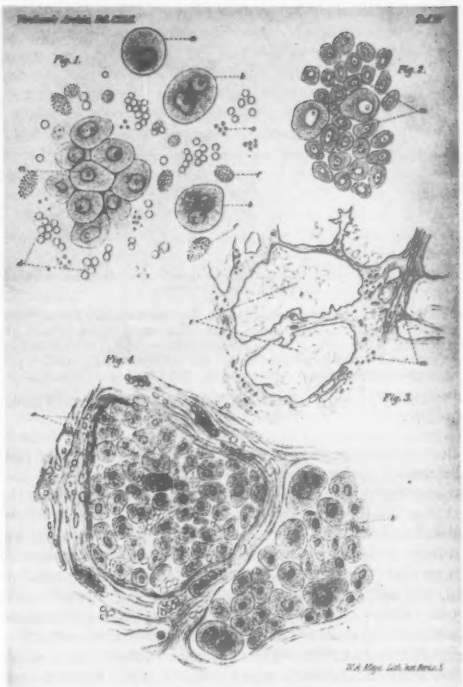


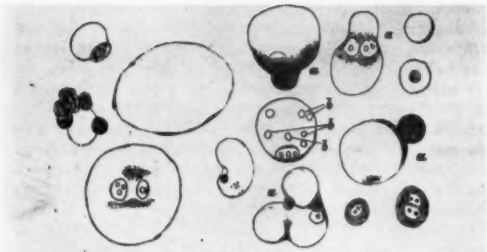
FIG. 10. From Betschart (1895). Translation of original legend: "Fig. 1. Fresh sputum. Enlargement 275 × .a) Mononuclear large round cell. b) Multinucleated round cells. d) Red blood corpuscles. e) Free fat drops. f) Granules containing cell. m) Large group of cells. Fig. 2. Fresh sputum. Enlargement 275 × .m) Large cell group. Fig. 3. Lung section stained with Borax-Carmin. Low power enlargement. s) Carcinomatous tissue. m) Lung tissue. Fig. 4. Lung tissue, enlargement 275 × .a) and b) Alveoles filled with mono- and multi-nucleated cells."

Historically, there was only a slight difference in time between the first cytological diagnosis of malignancy in serous effusions—Lücke and Klebs (166), 1867, and in sputa-Beale (12), 1860—despite the fact that the latter material has always been more readily available for study. Illustrations in a paper of Quincke (212), a clinician in Kiel, Germany (1882), demonstrate the characteristic cellular patterns of an adenocarcinoma. Also, Quincke (211) reported in 1875 on the difficulties in examining fluids containing chyle and mentioned the possibility of ether extraction.

The list of authors of early publications on the cytological examination of serous effusions contains well-known names, such as Paul Ehrlich (55, 58). He was not only concerned with details of cytoanalysis (55, 58), but improved the technical procedures significantly by introducing and applying new staining methods (56, 57, 58) in clinical cytology. Other famous authors were Rieder (222), who first recommended an ether-alcohol mixture for the fixation of smears, and Widal (273, 274) and Ravaut (214), who gave the basic principles on the cytological patterns of tuberculous effusions and introduced the term "placards endotheliaux." This term reflects very precisely the tendency of mesothelial cells to grow and group in single layers.

In a very comprehensive bibliography Köniğer (142) has listed most of the European publications on cytology of serous effusions up to 1908.

It was also at the end of the 19th century that textbooks dealing with laboratory and microscopic techniques contained special chapters dedicated to cytodiagnostic methods,



Krebszellen aus Ascitesflüssigkeit bei Carcinoma ovarii et peritonei. Die Zellen zum Theil in hydropischer Degeneration; a a Gruppen von 2 und mehr Zellen; b b Tröpfchen, die sich durch Jod blaurosa färben.

FIG. 11. From Quincke (1882): Translation of the original legend: Cells from the ascitic fluid of a carcinoma ovarii and pertitoneii. A section of the cells shows hydropic degeneration; aa) Groups of two and more cells; bb) Drops which stain a pale pink color with iodine.

Reviewer's remark: This is an instructive illustration of signet-ring cells.

including those applied in chest diseases. In addition to critical remarks, such as those of Wethered (272), there were positive and instructive contributions, such as those of Beale (13) and Friedlander (71). Also, Friedlander (71) gave a most precise definition of the interrelationship between histology and cytology, a definition which is still true today. Concerning benign and malignant lesions of the female genital tract, he said, "in carcinoma uteri, one will find suspended in the liquid, cellular elements originating from the carcinomatous ulceration and even tissue particles whose microscopic structure will help to establish the diagnosis. However, in doubtful cases, when the question arises whether or not there is a carcinoma or a benign erosion, the cell examination alone will not be sufficient. In such cases, often the excision of a small piece of tissue has to be performed and its histologic examination will confirm the diagnosis."

It was in the early years of the 20th century that the application of the cell block technique found wider use for the examination of serous effusions (128, 168). This period was followed by attempts to apply vital staining methods in the cytodiagnosis of fluids. The most successful and important contribution in this respect was made by Quensel (209, 210) with his cadmium-methylene blue-sudan technique in 1928. Besides thoroughly studying the morphology of mesothelial cells, Quensel (209, 210) was concerned with investigations of the role of the nucleolus in benign and malignant cells. Extensive measurements of nucleolar diameters convinced him that the nuclear:nucleolar ratio is a very valuable criterion for differentiating cancer cells from those of other origin. In this respect, he had already noted a difference in the appearance of carcinoma and sarcoma cells Usually, he states, the latter will not show enlarged and bizarre single or few nucleoli but a larger number of small nucleoli scattered all over the nucleus. Recently, Quensel's impressions were confirmed by Siering and Aderhold (244) using another vital examination technique: the phase contrast microscope. Figure 12 shows an original photomicrograph by Quensel, demonstrating the distinct staining of nucleoli in his cadmium-methylene blue-sudan technique.

Later investigators, such as Zadek (291, 292) and Karp (132) also using Quensel's technique have perhaps overemphasized the importance of the nucleoli pattern in cancer diagnosis. Karp (132), for instance, using measurements of nuclear and nucleolar areas, found no overlapping of values between mesothelial and tumor cells at all (See Table I.)

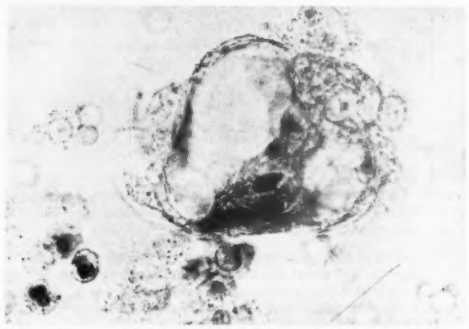


FIG. 12. From Quensel (1928). Translation of original legend: Carcinosis peritonei. Ascites of December 1st, 1927. Microphoto, 1 hour post-puncture. Vacuolized cells with very large nucleoli. In addition mesothelial cells, erythrocytes and leukocytes. Enlargement 500×.

TABLE I

NUCLEAR-NUCLEOLAR RATIO OF MESOTHELIAL AND TUMOR CELLS BY 3 DIFFERENT AUTHORS
(adapted from H. Grunze, 1955)

Enclosed in blocks overlapping values between mesothelial cells and tumor cells.

| | | |

In addition to pictures taken from vital stained specimens, the previously mentioned work of Zadek (292) contains beautiful drawings of Giemsa preparations and a complete bibliography, listing most of the publications up to 1933.

After a period of twenty years, since the last publication of Hampeln (100) in 1919, several remarkable papers on the subject of sputum cytology were published in Great Britain. The names of Roodhouse Gloyne (75, 76), Barret (11), Gowar (80), Dudgeon and Wrigley (51) should be mentioned. The latter used a technique originated in 1927 by Dudgeon and Patrick (50). The originators themselves described the principle of this new technique, which provides good transparency of smears rich in mucus, as a "wet film method," probably a precursor of the now widely applied ether-alcohol wet fixation of the Papanicolaou technique. Dudgeon and Patrick (50), using a mixture of alcohol, mercuric chloride, and acetic acid as a medium for the "wet film fixation," applied this procedure first in biopsy imprints for rapid diagnosis. The results with this technique as obtained by Roodhouse Gloyne (75, 76), Gowar (80), Barret (11) and Dudgeon and Wrigley (51) were quite satisfactory. The latter, for instance, yielded positive results in 68% of their 38 patients with cancer.

Not as satisfactory were the sputum examination results of Wuhrmann (290), who used a cell block technique simultaneously. Foot (66) and Zemansky (294) in 1929, also basing their investigations of sputum examination on the cell block technique, interestingly expressed the same skepticism.

Furthermore, Wuhrmann (290) mentions in this paper his experience with transthoracic needle biopsy of the lung. The first report of a large series of diagnostic needle biopsies of the lung, with special reference to tumor diagnosis, was published in 1934 by Martin and Ellis (170). Beginning with Leyden (153) in 1883 this procedure was usually limited to cases of pneumonia. In 1887 Krönig (148) (Berlin) was probably the first who used this technique in a single case to diagnose a malignant tumor of the lung. A comprehensive bibliography on the subject of needle biopsies of the lung appeared in a paper by C. R. Woolf (283).

Probably one of the most important contributions in clinical cytology of chest diseases was made by Wandall (269) in Copenhagen in 1944. His *Study on Neoplastic Cells in Sputum*, although published one year after the famous monograph of Papanicolaou and Traut, was started independently in 1941. Despite some technical improvements since that time, Wandall's results are still comparable to those obtained today. Using a modification of the Dudgeon and Patrick (50) "wet film technique," he obtained 84% positive results in the diagnosis of carcinoma by sputum examination in a series of 100 cases. In 62 cases out of 75 Wandall was able to determine the histological type of the tumor by cytological means. The risks of false-positive reports in chronic inflammatory diseases such as chronic pneumonia and tuberculosis are thoroughly discussed and illustrated by him. The optimistic as well as critical attitude of this instructive monograph by Wandall (269) served as a useful basis for further investigations in lung cytology.

## III. STAINING METHODS IN CLINICAL CYTOLOGY OF CHEST DISEASES

This section will be reserved for a few remarks on the advantages and disadvantages of the various methods presently used in cytodiagnosis. Some details on the collection and processing of specimens will be discussed in the following paragraphs.

There is little doubt that the Papanicolaou stain (197) is now accepted as the routine method of choice for cytological examinations. Only a few authors still prefer the familiar

TABLE II*

ADVANTAGES AND DISADVANTAGES OF VARIOUS STAINING METHODS

| Positive Properties | Pappenheim, Giemsa, Wright Stain | Papanicolaou Stain | Dudgeon & Patrick Wet Film Technique | Quensel Stain | Phase Contrast | Floures-cence |
|---|---|---|---|---|---|---|
| Qualification for Documentation | +++ | +++ | +++ | – | – | – |
| Quick processing and staining of smears | +(+) (++ Giemsa wet film technique) | – | + | ++ | +++ | ++ |
| Transparency of smears and preparations | – (++ Giemsa wet film technique) | +++ | +++ | +++ | +++ | ++ |
| Minimum of cell shrinkage | + (++ thin smears) | ++ | ++ | +++ | +++ | +++ |
| Brilliance of colors | +++ | ++ | + | ++ | +++ | +++ |
| Differentiation of Nuclei | ++ | ++ | ++ | ++ | +++ | + |
| of Nucleoli | ++ | ++ | ++ | +++ | +++ | 0 |
| of Cytoplasm | +++ | ++ | 0 | +++ | +++ | + |
| Minimum of mechanical cell damage during smear preparation | 0 | + | + | +++ | +++ | +++ |
| Qualification for large scale screening | + | +++ | ++ | – | – | – |

+ + + = excellent
+ + = good
+ = sufficient
0 = not sufficient
– = lacking

* Adapted from H. Grunze (1955)

hematology stains, such as the Wright, Pappenheim, Romanowsky, or Giemsa stain (44, 160, 161, 252, 253). The reviewer, using a modified Giemsa wet-film technique for special cases, suggests that it is useful to have information from both the Papanicolaou and the Giesma stain in systemic disorders of the blood, the lymphatic and the reticuloendothelial system. Involvement of the chest region by these diseases is not rare.

The phase contrast microscope, widely and successfully used in research, has not yet received similar appreciation in practical use for lung cytology. It is perhaps the impossibility, or the extreme difficulty of getting permanent preparations for documentation and for re-examination with this method which prevented its application on a larger scale. The same might be true for vital staining methods, such as Quensel's cadmium-methylene-blue-sudan stain or the acridine-orange fluorescence technique. Wittekind (280) recently has recommended a combination of both the phase contrast and the fluorescence technique for a quick low-power screening of smears. Other studies with vital methods containing instructive illustrations have been published by Siering and Aderhold (244), Lüdin (167), Reitter (218), von Albertini (1) and Riegel (223). Only the latter two are especially dedicated to the examination of chest material. The former are dealing, preferably, with general tumor cytology and comparison of different techniques. In the reviewer's opinion it is too early to make final statements on the value of these vital methods in chest cytology. In the experience of the reviewer and other authors the advantages of phase contrast microscopy are most obvious for immediate diagnosis, as during surgery; however, it is still uncertain whether or not microphotographs will be generally accepted for documentation, as suggested by Wittekind (280), Riegel (223), and others. If so, there will still remain the impossibility of re-examining such vital preparations Table II demonstrates briefly some of the advantages and disadvantages of the various staining methodss.

## IV. PRESENT STAGE OF CLINICAL CYTOLOGY IN CHEST DISEASES

### A. Cytology of Sputum and Bronchial Secretions

Special monographs on this subject have been published by Wandall (269), Farber et al. (61), Hartmann (103), Grunze (88, 89), and Strupler (259). Detailed chapters dealing with cytodiagnosis in chest diseases are a part of the monographs on Clinical or Exfoliative Cytology by Ruth Graham, et al. (82), Papanicolaou (198), Lopes-Cardozo (161) and Maria Kawecka (133). A review article by Ruth Graham (83), paying special attention to the American literature, gives a comprehensive survey on the same subject up to 1954.

#### 1. Collection and Processing of Specimen

Although realizing the fact that experienced investigators, such as Farber et al. (61), have obtained better results by using sputum specimens rather than bronchial secretions, it seems to be fair to state that both materials have their advantages. The decision as to which specimen should be used routinely and which used in special cases is dependent upon the laboratory and endoscopic facilities of the individual clinic. Touch smears, aspirations or scrapings from carefully selected areas of the bronchial system might provide a specimen less contaminated with non-diagnostic cells (16, 59, 64, 110, 111, 112, 139, 180, 202). This will save a great deal of time during the screening procedure. Furthermore, scraping, as described by Delarue et al. (42, 43), Dijkstra (45), Huizinga (121), Lopes-Cardozo and Janssen (162) and Brandt (24), will result sometimes in obtaining diagnostic material even from lesions beneath the intact mucosa when spontaneous exfoliation is not possible. These advantages of the bronchial secretions are still true today, although the quality of sputum

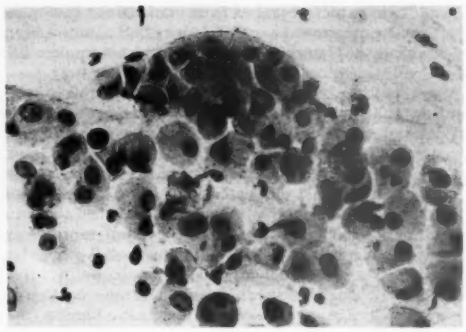


FIG. 13. Sheet of alveolar cell epithelia obtained from a 32 year old control subject after inhalation of an hypertonic saline solution for 15 minutes according to Bickermann et al. (20). A report of Doris Rome (225) confirmed that the inhaled salt fumes do not cause any marked cell damage which would render the analysis of cells more difficult.

specimens has been markedly improved by applying inhalation procedures with hypertonic saline solutions (20).

In the reviewer's experience (88, 89) the diagnostic value of bronchial secretions increases significantly when bronchoscopy is performed under general anesthesia by intubation and artificial respiration. On the other hand, there is still the drawback that bronchoscopy is inconvenient for the patient, therefore causing difficulty in collecting a specimen as often as one would like. How significantly repeated examinations will improve the results was pointed out relatively early by Farber et al. (61), who stated, "malignant cells were found in 59% of the cases by examining an average of three specimens per patient. However, positive cytologic diagnoses were made in 87% when five sputum specimens were examined." This indicates that the sensitivity of the cytologic technique will increase as a function of the number of specimens examined.

Furthermore, there is some evidence that repeated examinations of sputum specimens might be superior to single bronchial washings in peripheral lesions (61, 126, 251). (See Table III).

TABLE III

DIAGNOSIS OF PERIPHERAL LESIONS

| Site of Lesion | Cytology | | Bronchoscopy |
|---|---|---|---|
| | Bronchial Aspirations | Sputum | |
| Right Lung:.... | Positive 2<br>Negative 9 | Positive 15<br>Negative 10 | Suspicious 5<br>Negative 16 |
| Left Lung:..... | Positive 1<br>Negative 9 | Positive 15<br>Negative 11 | Suspicious 4<br>Negative 16 |

(From Farber, et al. (61): Cytologic Diagnosis of Lung Cancer. Springfield, Ill., 1950, C. C. Thomas.

In addition to the usual expectorant procedures (increased water intake, potassium iodine medication and recently hypertonic salt solution inhalations), Sirtori (246b) even applied local radiation and intramuscular trypsin medication in order to increase the exfoliation rate of cells from lung lesions. There seems to be no confirmation at the present time that this procedure has been accepted in other laboratories.

As yet, attempts to enrich the cellular content by mucus digestion, Farber (62), Umiker (264), have failed to improve results significantly. Adenocarcinoma cells and cellular elements of undifferentiated tumors were selectively severely altered (263). However, improved methods (203a, 203b) might change the outlook. At the present time the reviewer himself

still prefers obtaining fresh unfixed specimens whenever possible in order to make a macroscopic selection of the material according to Gowar (80) and others (96, 126). In this respect fixed material has some disadvantages. However, whenever there is significant time lapse between the collection of material and its processing, there seems to be no alternative but to accept fixed material or covered slides (Papanicolaou-197).

Several authors believe that sputum paraffin cell block sections provide better results than the smear technique (39, 129, 131, 246, 277, 294). Similar to pleural effusions, this might be true in the presence of organoid tumor formations or even small cell groups but not if only single cells, especially those of the small-cell type, are present. In this respect the reviewer shares the opinion of Farber et al. (61), and Richardson et al. (220) when giving preference to the smear technique as a routine procedure. However, application of both the section and smear technique in well-staffed laboratories might be helpful in some cases due to the fact that serial sections permit screening of a larger part of specimens within a limited time.

2. Results in Cancer Diagnosis

*a. Positive Correct Results in Cancer Diagnosis:*

There is a marked variation in the results of various investigators during the last ten years (See Table IV) It is probable that differences in the qualification of the listed authors are far less important than differences in their working conditions; for instance, the quality of bronchoscopic examinations and of the secretions obtained thereby and the source of these specimens (whether in-patients or out-patients) are important factors. The out-patient group will show less favorable results due to more difficulties in obtaining repeated, adequate specimens.

It seems to be likely that the average fraction of positive correct results in the diagnosis of bronchogenic cancer ranges under favorable conditions at the present time between 70–85% when using both sputum and bronchial secretions.

TABLE IV

POSITIVE CORRECT RESULTS IN CANCER DIAGNOSIS BY DIFFERENT AUTHORS

| Reference | No. of Pulmonary Cancer | Diagnosed Accurately % |
|---|---|---|
| Hampeln (1919) (100) | 25 | 52.0 |
| Dudgeon and Wrigley (1935) (51) | 38 | 68.0 |
| Gowar (1943) (80) | 63 | 64.0 |
| Wandall (1944) (269) | 100 | 84.0 |
| Herbut and Clerf (1946) (112) | 57 | 80.2 |
| Liebow et al (1948) (157) | 49 | 43.0 |
| Woolner and McDonald (1949) (286) | 147 | 68.0 |
| Kucsko and Portele (1949) (149) | 124 | 75.0 |
| Watson et al. (1949) (270) | 236 | 60.0 |
| Hengstmann and Wittekind (1950) (110) | 22 | 86.3 |
| Herbut (1953) (113) | 540 | 88.3 |
| Jackson et al. (1951) (123) | 100 | 61.0 |
| Heckner (1952) (107) | 78 | 20.0 |
| Umiker (1952) (262) | 20 | 70.0 |
| Iversen (1953) (122) | 124 | 46.0 |
| Hartmann et al. (1953) (104) | 134 | 71.6 |
| Hengstmann (1953) (111) | 72 | 72.1 |
| Spjut et al. (1955) (251) | 501 | 57.8 |
| Sirtori (1959) (246b) | 150 | 92.5 |
| Kahlau (1958) (129) | 547 | 67.0 |
| Garret et al. (1958) (73) | 50 | 58.0 |
| Kawecka (1959) (134) | 595 | 87.0 |

Further reference to the overall results of sputum cytology is given in articles of Ruth Graham (83, 84), Grunze (89), Hartmann (103), Strupler (259), Maroun (169) and others (36, 37, 43, 65, 72, 109, 116, 143, 174, 176, 193, 221, 258, 271, 282, 284, 286).

Another way to prove the value of cytology of sputum and of bronchial secretions is to compare histological and cyto logical results and to evaluate how many cases could be diagnosed only by means of cytology (Table V).

TABLE V

| Reference | No of Tumors | Morphological Diagnosis possible only by Cytology |
|---|---|---|
| Wandall (1944) (269) | 100 | 73% |
| Herbut and Clerf (1946) (112) | 57 | 12 cases in which bronchoscopy was negative |
| Woolner and McDonald (1949) (284) | | 29 of 74 patients who were undergoing thoracotomy |
| Watson et al. (1949) (270) | | 7 of 26 resectable cases |
| Farber et al. (1950) (61) | | 39% of the cases in which bronchoscopy was negative |
| O'Keefe et al. (1950) (188) | 307 | 30.6% in which bronchoscopy was negative |
| Jackson et al. (1951) (123) | 100 | 18% |
| Herbut (1953) (113) | 540 | 57.4% |
| Link (1952) (158) | 52 | 37% |
| Bitschin (1953) (21) | 57 | 15 cases in which bronchoscop was negative |
| Jennings and Shaw (1953) (126) | | 64 resectable cases |
| Spjut et al. (1955) (251) | 501 | 29% of operable cases |

Spjut et al. (251) stated, on the basis of their series, that positive biopsy by bronchoscopy lessened the chance of surgery, whereas positive cytology did not. Iversen (122) has found a little higher rate of positive cytology in advanced cases (47%) than in resectable patients (42%). Spjut et al. (251) and Jennings and Shaw (126) could find no difference at all. Both groups pointed out that despite a lower rate of positive results in peripheral lesions compared to those of more central location, cytology was very valuable in defining such peripheral tumors.

The figures of Spjut et al. (251) would indicate that there is a certain relationship between the size of a given lesion (exfoliation rate?) and the rate of positive results. However, there is no linear correlation. Small lesions and medium sized tumors of four to seven centimeters, for instance, have no favorable results, whereas those of two to three centimeters do produce favorable results This study lacks, in the reviewer's opinion, the subgrouping of histologic tumor types

and of bronchographic findings, in addition to the correlation of the size of the lesion.

A proper preexamination of the cases and thorough instruction of the patient by the clinician on how to produce a good specimen will be very valuable in increasing the efficiency of the cytologic method. Otherwise, figures as obtained by Hampson (101) will be representative; he found that from 855 submitted specimens of 351 patients, only 42 patients (11.9%) turned out positive or suspicious.

*b. False Negative Results in Cancer Diagnosis:*

Since false positive results are not included in Table IV, false negative diagnoses may be calculated by the difference between overall number of tumors and the number of correct positive results as listed in that table. In a special study Umiker (263) reported in 1957 on "wrong negative results." He mentioned as primary causative factors for wrong negative results:

1. absence of a communication between the neoplasm and bronchial lumens,
2. stenosis of bronchi,
3. misinterpretation of smears.

Secondary factors are, according to Umiker:

4. location of tumor,
5. histologic type of neoplasm,
6. quality and quantity of specimens,
7. presence of secondary inflammation, and
8. the type of specimen (sputum or bronchial aspirate).

Points 1 and 2 are of overwhelming importance; misinterpretation of smears ranges in our experience behind point 6. Point 3 seems to summarize the difficulties of items 5 and 7.

Similar studies on the causative factors of false negative results have been reported by Buffmire and McDonald (30) and Spjut et al. (251).

In general, "false negative results" are usually not "false" from the point of view of the cytologist; the smears simply do not contain tumor cells due to bronchial stenosis or lack of exfoliation.

The tendency for exfoliation was studied experimentally by Appel and Bronk (2). For clinical purposes, it is important to note that the most common cancer in clinical treatment, the squamous cell cancer, has a high tendency to exfoliate, as mentioned by several authors (61, 67, 69, 70, 122). For illustration see Table VII.

At the present time any effort to decrease the fraction of false negative results should start with further attempts to improve the quality of the collected specimens.

*c. False Positive Results in Cancer Diagnosis:*

Despite their low incidence in experienced laboratories, false positive results present a very serious problem. Although there are various reasons and excuses which might explain false negative results, there is only one causative factor for the occurrence of false positive results: the misinterpretation of smears by the cytologist! His mistake might have unfortunate consequences for the individual patient, since cytology claims to provide a morphological diagnosis when biopsy is not possible (103, 270). As a result of this, unnecessary chest surgery or radiation might be carried out. Looking for the reasons which mislead even experienced examiners one will find in most instances cellular changes in chronic inflammatory disorders (Table VI).

TABLE VI

FALSE POSITIVE RESULTS IN CANCER DIAGNOSIS
(Results obtained without detailed clinical information)

| Reference | No. of overall patients | Clinical Diagnosis or probably underlying cyto-histologic changes, causing the false positive result |
|---|---|---|
| Dudgeon and Wrigley (1935) (51)..... | 270 | 1× Chronic sinusitis<br>1× Polyps of nasal mucosa |
| Wandall (1944) (269). | 193 | 3× Chronic pneumonia (atypical histiocytes)<br>3× Tuberculosis (epitheloid cells) |
| Bamforth (1946) (7).. | 113 | 1× Bronchiectatic cavern with papillary mucosa formations and exfoliation of of basal cells |
| Liebow et al. (1948) (157)..... | 131 | 4× Bronchiectasis<br>1× Lung abscess |
| McKay et al. (1948) (180)..... | 170 | 2× Tuberculosis<br>1× Bronchial polyp |
| Watson et al. (1949) (270)..... | 1200 | 3× Bronchiectasis<br>7× Not mentioned |
| Woolner and McDonald (1949) (284, 286) | 2188 | 1× Tuberculosis<br>1× Bronchiectasis<br>1× Multiple lung infarcts followed by chronic pneumonia and squamous metaplasia of drainage bronchi<br>1× Pneumonitis (was interpreted as small cell carcinoma, possibly misinterpretation of cuboid alveolar cells) |
| Kjaer et al. (1949) (138)..... | 125 (selected cases with difficult differential diagnoses) | 10× Bronchiectasis and chronic pneumonia<br>7× Lung abscess<br>2× Lung infiltration<br>1× Silicosis and Tuberlosis<br>2× Empyema<br>3× Pleuritis exudativa |
| Farber et al. (1950) (61)..... | 1254 | 1× Lung abscess (crowd of histiocytes)<br>1× Squamous metaplasia |
| Jackson et al. (1951) (123)..... | 270 | 2× Tuberculosis<br>1× Lipoid pneumonia with bronchiectasis (histology: squamous metaplasia) |
| Frühling and Wachenheim (1952) (72)... | 138 | 4× False positive results caused by "irritated" alveolar epithelia or by lack of cell groups in the sections (the authors used a cell block technique) |

TABLE VI—*Continued*

| Reference | No. of overall patients | Clinical Diagnosis or probably underlying cyto-histologic changes causing the false positive result |
|---|---|---|
| Hjelt (1952) (116).... | 580 | 2.6% in sputum<br>3.3% in bronchial washings |
| Iversen (1953) (122).. | 624 | 1.4% |
| McDonald (1954) (177)..... | 1500 | 1.6% |
| Kawecka (1959) (134) | 1127 | 24 cases where either false-positive diagnosis was given or a false positive suspicion was raised showed 10× squamous metaplasia and 22× bronchitis; a few had bronchiectasis |
| Grunze, up to 1959 (working with full information on clinical data)..... | 2800 | 1× Squamous metaplasia<br>1× Necrotic material<br>1× Chronic pneumonia (histology "atypical proliferation" of alveolar epithelium)<br>1× Alveolar cell polymorphism in lung fibrosis |

The figures in Table VI should encourage intensified studies of cellular patterns in inflammation and granulomatous conditions. This would not only improve the differential diagnostic value of cytologic reports and economize cytology in chest diseases by reason of less frequent "negative results," but also would help to keep false positive results at a minimal level. At present a percentage of 0.5–2 of false positive diagnoses might be considered as inevitable when working without detailed clinical information (84, 282, 287). Although clinical information does not help in cell analysis itself, it makes the examiner alert for those situations in which danger of misinterpretation might occur. Furthermore, in difficult cases it will help in suggesting special procedures for the collection and processing of material, if necessary. However, the fear of false positive results and the trend toward reliability should not predominate so strongly that the result of this would be a lack of any sensitivity of the method. Reliability and sensitivity must be in balance. Even the histological examiner during exploratory thoracotomy risks being wrong from time to time, as was recently reported for instance by Becker and Knothe (15).

In addition to the experience of the cytologist (122, 269, 285), there is little or no help in the differentiation of benign "active cells" from malignant elements, not even by special staining methods.

These authors have proved in parallel studies, or when analyzing their early and their late results, that additional experience decreased the percentage of false positive results. Maria Kawecka (134), paying special attention to those cases, showing squamous metaplasia,* mentioned the following criteria for the recognition of metaplastic and malignant squamous cells. The former will usually show:

(1). more frequent appearance as single cells than in cell groups,

(2). a varying degree of enlargement of round nuclei,

(3). an even chromatin distribution and "nuclear chromatin structure,"

(4). only very rarely multinucleated or bizarre giant cells

Maria Kawecka states further that the nuclei may be also "very dark." However, according to our experience (92) the difference in the chromatin content of metaplastic and tumor cells seems to be the only criterion relatively reliable when studying a number of cells in borderline cases. Furthermore, we did not feel that the criterion mentioned under point 1 by Maria Kawecka was helpful in difficult situations.

The discussion on spindle cells by Ruth Graham (85), Koss (147), von Haam (95), Y. S. Song (250) and this reviewer in Acta Cytologica, Vol. II, No. 2, provides further information on the subject. Also the monographs of Strupler (258) and Hartmann (103) and papers of Delarue and Orcel (43), Bergman and co-workers (17), Koss (145, 146), Kolyaditskaja (144), Carabello (31), Chipps and Kraul (34) and Hjelt (116) illustrate diagnostic difficulties in inflammatory disorders. Reports of Hannah Peters (201), Toaff and Bromberg (261) and Johnson et al. (127), describing the cellular patterns of benign and malignant disorders of the squamous epithelia from the mouth, pharynx and esophagus, might be helpful when differential diagnostic problems are caused by contamination with secretions from these sources. The same aid might be given by the very thorough studies of Pfaltz and Probst (202, 202a, 202b) as far as contamination with secretions from the nose and the sinuses are concerned.

There are at the present time no special studies available on the difficulties which might arise by misinterpreting alveolar cuboidal cells. Mainly, this is due to the fact that the existence of alveolar epithelium under physiological and even under pathological conditions was denied by the overwhelming majority of Anglo-Saxon and French authors. Heckner (107) described these cells as "pseudo tumor" cells; the reviewer himself (88, 89) has studied the morphology of alveolar epithelia by both methods (bronchial secretions and lung aspirations). He expresses the opinion that the so-called "Pap cells" in some instances might not only be of squamous metaplastic but also of alveolar cuboidal cell origin.

Concluding the thorny problem of false positive reports in cancer diagnosis and introducing the chapter on cytodiagnosis of inflammatory and granulomatous disorders, a remark made by Hampson (102) at the Congress in Brussels in 1957 may be cited: "It is a truism that sound knowledge of the normal is the only firm foundation upon which to build a knowledge of the pathological. Nevertheless, this can hardly be repeated too often or too clearly as far as exfoliative cytology is concerned, for the subject has suffered and still suffers from lack of knowledge of the possible variations of normal cells and more particularly from an incomplete picture of the origin and modes of production of these cells."

### 3. Cytodiagnosis of Inflammatory Disorders, Granulomatous Conditions, Benign Tumors and Malignant Disorders of the Lymphatic and Reticuloendothelial System

#### *Sputum*

In sputum the macroscopic and bacteriologic findings generally are of major importance and the cytologic results have only minor value in the diagnosis of inflammatory and granulomatous disorders. However, there are a few exceptions which should be mentioned.

Cytology helps to control the quality of "washed sputum" for bacteriologic purposes as described by Mulder (184) and cited by Bucher (29). Sputum might show "CCP" cells, see Papanicolaou (196), in virus infections similar to earlier reports of Hilding (114) as well as in bacterial infections or malignant disorders. "Pap cells" and squamous metaplastic cells might indicate more chronic disorders. Epitheloid cells and typical Langhans giant cells will occur sometimes in sputa of

* Histological studies on the questionable relationship between squamous metaplasia and squamous cancer have been performed and reported by several authors (5, 231, 265, 279).

tuberculous patients (88, 269). However, the diagnosis has to be confirmed by bacteriologic studies. One seldom finds such typical Langhans giant cells and epitheloid cells, as well as caseation, that confusion with non-specific alveolar cells and histiocytes is completely ruled out. But even when epitheloid cells or Langhans giant cells have been found, both usually lacking dust phagocytosis, one has to consider that these elements are not only typical for tuberculosis but also might be found in other granulomatous disorders-experience with lymph node puncture, cited by Grunze (88). As a rule the findings in sarcoidosis of the lung are absolutely negative. The scar-character of the lesions apparently prevents any exfoliation of specific cells. Only in one case with superimposed infection did we find, in our twelve year's experience, sheets of epitheloid cells. There are no further reports in the literature (to the reviewer's knowledge).

Hemosiderin macrophages have little diagnostic value in heart diseases, even when they occur in abundance and even after occult lung bleeding has been ruled out, as compared with other diagnostic tests. Beyond the age of thirty the amount of alveolar cells in sputum continuously increases even in healthy people (94). Groups of alveolar cells of all types will occur in atelectasis, dystelectasis and the various diseases accompanied by lung fibrosis.

A specific diagnosis by sputum examination might be possible in lipoid or oil pneumonia. Technical advice and help in critical evaluation of such cases may be obtained from reports of Farber et al. (60), Losner et al. (164), Jampolis et al. (124), Nathanson et al. (187), Janes (125) and Grunze (88). Naturally, fat dissolving fixation procedures should be avoided.

There are no reports available at present on cytodiagnosis of Gaucher's or Niemann-Pick's disease of the lung. However, even in large departments of pathology, these conditions are extremely rare.

Sputum findings in lymphosarcoma and Hodgkin's disease have been described by Dawe et al. (40), Florentin et al. (64), Delarue and Orcel (43), Papanicolaou (198), Grunze (88, 89) and Efskind and Wexels (54). In Hodgkin's disease a finding of reticulum cells alone is not diagnostic. These cells also might occur in inflammatory conditions, mainly when there is a lack of bone marrow reaction, as after radiation or in myelofibrosis. Evidence of Sternberg-Reed cells, however, is diagnostic.

Up to the present time benign connective tissue tumors could not be diagnosed by exfoliative cytology. Those of epithelial origin (bronchial-adenoma) were diagnosed in most instances in bronchial aspirates. Woolner and McDonald (287, 288) stated relatively early that sputum examination is of little value in the detection of bronchial adenoma or cylindroma. Wandall (269) misdiagnosed one case of bronchial adenoma in sputum as being malignant, and Spjut et al. (251) misinterpreted adenoma cells three times as malignant, once as undifferentiated cancer, once as squamous cell cancer and once as an adenocarcinoma. Papanicolaou (198) describes the typical features of adenoma cells with eccentric location of the nucleus in cells of the basal cell type in his atlas.

### *Bronchial smear, aspirate and washing*

Contamination with secretions from different sources and quick autolysis of bronchiolar epithelia do not permit any detailed conclusion concerning the condition of the bronchial mucosa by means of sputum cytology. Specimens carefully taken during bronchoscopy, however, will provide such information.

The basic rules in differentiating the various forms of bronchitis, such as hypertrophic, atrophic, bacterial, abacterial, acute, chronic and necrotizing bronchitis, have been outlined by Pfaltz and Probst (202a, 202b), Strupler (259), Hartmann (103) and Grunze (88, 89). In the reviewer's opinion the results thus obtained are comparable with those found by histologists (216). For example, cytology confirms that the early stage of chronic bronchitis is, in many cases, a functional one with an abundant number of goblet cells.

Cytologic preparations compared to biopsies have the disadvantage of not showing the structure of the bronchial mucosa, particularly that of the deeper layers. Even after scraping, there only will be an exfoliation of basal cells. On the other hand, cytologic smears have the advantage of being representative of a wider area of the bronchial mucosa. The latter might be important when it is desirable to get some information about the alterations in the periphery. However, there are two difficulties: It requires extensive training, experience and imagination on the part of the examining cytologist to reconstruct the probable tissue alterations by quantitatively and qualitatively analyzing the exfoliative picture. Secondly, a skilled bronchoscopist who uses standardized methods for obtaining the material, is required. It is very important, for instance, not to use any absorbent material such as cotton for obtaining the smears, but to use a non-absorbent tissue, such as nylon. We prefer performing bronchoscopy under general anesthesia, as outlined by Brandt ((24). Bronchoscopy done in this manner may help us in obtaining useful specimens for future research projects in "chronic bronchitis." The various morphological features of bronchial epithelia, such as "Kümmerformen" (miserable forms) in atrophic bronchitis, can be found in the papers of Pfaltz and Probst (202a, 202b).

Bronchoscopically obtained specimens will also offer an opportunity to study squamous metaplasia in various conditions (92, 103). Changes due to radiation have been described in detail by Strupler (259) and Koss (146, 147).

Recognition of cuboidal alveolar cells in chronic inflammatory disorders with "atypical proliferation" (137, 156) is more accurate in bronchial aspirates than in sputa.

Since autolytic cellular changes are less pronounced in bronchial secretions than in sputa, the other inflammatory and granulomatous disorders, as mentioned under "sputum," will also be more accurately diagnosed. An example is the recognition by cytologic means of lymph node perforation into the bronchus in childhood tuberculosis (88, 89).

Also significant and important are the more favorable results in the diagnosis of bronchial adenoma when using bronchial smears or aspirates instead of sputa. Frequency of positive findings as well as accuracy in determining the histological type are improving.

One of the reasons for better results in the diagnosis of bronchial adenoma might be the ability to induce exfoliation of adenoma cells by mechanically injuring the overlying bronchial mucosa (207). Important contributions have been reported in this regard by Swiss authors (21, 202a, 202b, 259). The difficulties in the differential diagnosis of bronchial adenoma and squamous cancer of the basal cell type have been discussed by Strupler (259) and Grunze (88, 89). Correlating histological and anatomical aspects in bronchial adenoma are outlined in a paper of McBurney et al. (175).

Due to a lower degree of autolysis, the recognition of disorders of the lymphatic and hematopoetic system is also safer in bronchial aspirates than in sputum. However, lung infiltrations by lymphomas or leukemic disorders are usually diagnostic problems only as to whether or not they are true tumor infiltrates or simply intercurrent inflammatory disorders. We have observed, to date, seven cases of Hodgkin's disease with involvement of the respiratory system and with positive cytologic diagnoses. In only one was the basic underlying diagnosis not known beforehand. In both lymphoma and leukemic disorders the parallel use of the familiar hematologic stains is most contributory. The correlative study of peripheral blood smears and bone marrow is also quite helpful. For instance, the rate of leukemic or sarcomatous cells in bronchial aspirates has to be significantly higher than in the peripheral blood.

Only when such additional information as mentioned above (hematologic stains and data and thorough clinical in-

TABLE VII

CANCER TYPE, POSITIVE RESULTS, DIFFERENCE BETWEEN RESECTABLE AND NON-RESECTABLE PATIENTS
(adapted from Iversen-122)

| | No. of Patients | Cytology Positive | Resectable Patients | Cytology Positive | Non-Resectable Patients | Cytology Positive |
|---|---|---|---|---|---|---|
| Squamous cell cancer | 152 | 76 (50%) | 94 | 45 (48%) | 58 | 31 (54%) |
| Adenocarcinoma | 30 | 8 (27%) | 21 | 7 (33%) | 9 | 1 (11%) |
| Anaplastic carcinoma | 63 | 25 (40%) | 20 | 4 (20%) | 43 | 21 (50%) |
| Miscellaneous tumors | 5 | 1 (20%) | 2 | 1 (50%) | 3 | 0 ( 0%) |
| TOTAL | 250 | 110 (44%) | 137 | 57 (42%) | 113 | 53 (47%) |

formation) is available, may a specific diagnosis of systemic disorders be made by cytologic means with any frequency. Otherwise the skepticism, as expressed by Dawe et al. (40), is fully justified.

4. Possibilities for Histologic Typing in Cytodiagnosis

One becomes aware of the surprising development of cytodiagnosis when the past and present problems are considered. Thirty years ago even experienced pathologists questioned whether or not it was possible to establish a diagnosis on the basis of isolated cells. Today the debatable point is the percentage of cases in which it is possible to determine cytologically the histologic type of a lesion.

As indicated in the introduction to this review, attempting to give a more specific diagnosis means improving the diagnostic and differential diagnostic value of cytologic reports. The late Dr. C. N. Foot (67, 69, 70) has earned special merit in this regard. His extensive investigations confirmed that Wandall's (269) results, with approximately 80% accuracy in tumor typing, were, and still are, representative, when observing the optimal results now obtained (61, 251). Less favorable results are reported by Jackson et al. (123).

There is general agreement that reports on the diagnosis of cornifying squamous cancers have the highest rate of reliability; whereas, reports on undifferentiated carcinomas and adenocarcinomas have lower rates. This distribution of frequency in the accurate typing of tumors by cytologic means parallels the frequency of the positive overall results (also when making a decision based on the Papanicolaou scale alone), because the histologic control of the cases which had positive cytodiagnosis will later show that the majority were of squamous cell type. In brief: the tumor type having the best chance to be diagnosed by means of cytology also has the best chance of being correctly typed. This holds true for both the good overall results of Jennings and Shaw 83.9% (199) and for the less favorable overall results of Iversen 44% (122, See Table VII).

As indicated in Table VII and as is known by the early published results of chest surgery centers, for example, Overholt et al. (189) and Brunner (27, 28), the differentiated squamous cell cancers not only have the best chance of resectability, but also the best survival rate. For this reason it is valuable for the clinician to obtain information on the histologic type of a lesion before surgery, since a risk might be taken in the treatment of a well-differentiated squamous cell cancer, but might not be taken in another type of cancer known to have a less favorable course.

Another situation where any attempt of the cytologist in giving a more specific answer is well appreciated by the clinician occurs in the differential diagnosis of metastatic lesions, since the search for a primary tumor can be very difficult.

In most instances, probably due to a long-lasting lack of bronchial drainage, the results in cytodiagnosis of metastatic lung lesions are less favorable than in primary lung cancer. Even large-sized metastatic tumors might not show any exfoliation. This fact has been known since Wandall's (269) and Farber's (61) studies and was recently pointed out again by Barbier (8) and Rosenberg et al. (229).

The latter have found that "correspondence of cytologic type with that of the suspected primary lesion helps to confirm the diagnosis of metastatic disease. Discrepancy in type from a suspected primary source may indicate that the pulmonary lesion is actually primary."

Summarizing, Rosenberg et al. (229) state: "The percentage of positive results from exfoliative cytology in metastatic diseases, although low (38% in their series), is encouraging."

Details on the cytologic patterns of the various tumor types, metastatic lesions as well as primary lung tumors, are available in the monographs mentioned in the introduction to this article and in the papers of Foot (67, 69, 70), Philps (205) and Pfaltz and Probst (202a, 202b).

Attempting to specify the tumor type, the following difficulties might occur:

(1). Lack of cell groups or too scant cellular material.

(2). Lack of qualitative cellular changes indicating special functions (keratinization, mucus production).

(3). Hydrophilic degeneration of cytoplasm simulating mucus production.

(4). Less frequent, but very troublesome is the occurrence of different cell types in adenocanthoma or in primary dual cancer of the lung with differen thistological types. (The latter

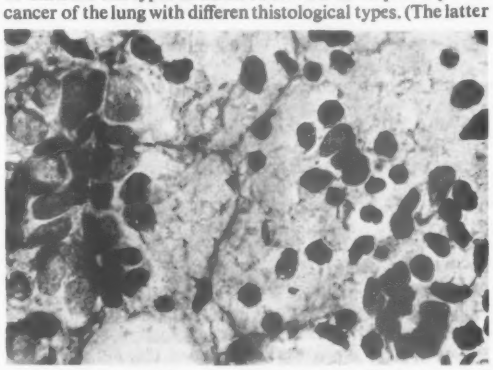


FIG. 14. Imprint from a surgical specimen. Both tumor cell types, oat cell carcinoma and squamous cell cancer of basal cell type, here in one microscopic field, were present in the bronchial aspirate and in the sputum before surgery and were diagnosed as such.

situation occurred once in our series of more than 400 lung cancer patients. The diagnosis was made correctly before surgery.) Data on the evidence of dual lung cancer are available in a paper of Robinson and Jackson (224).

(5). Occurrence of different cell types in undifferentiated small cell carcinomas containing partially insular-like differentiation into squamous cell cancer or adenocarcinoma.

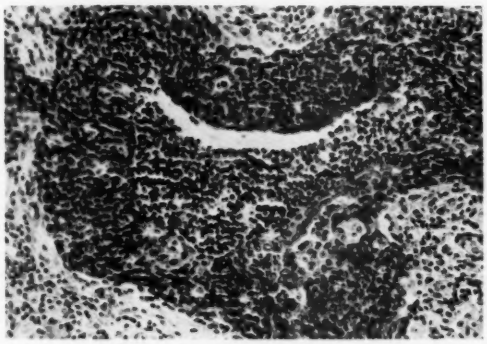


FIG. 15. Histological section of the case shown in Fig. 14.

(6.) Differentiation of small cell tumors (squamous cell cancer of basal cell type, bronchial adenoma, oat cell carcinoma, lymphosarcoma). In contrast to oat cell carcinoma, lymphosarcoma tumors show no tendency for autolysis and the cytoplasm of the cells stains typical azure blue in hematologic stains. Bronchial adenoma cells are fairly even in size. Their cytoplasm is gray in the Giemsa stain and their nuclei are round or oval, often located eccentrically (198). Nucleoli can be distinctive, but not as prominent as in the basal cell type of squamous cancer. Oat cell carcinoma cells usually do not show distinct nucleoli. Their hyperchromatic nuclei, although not significantly varying in size, show marked polymorphism in shape and, as a rule, only residuals of an ill-defined grayish cytoplasm or are absolutely naked.

*Differentiation between primary bronchogenic adenocarcinoma and alveolar cell carcinoma*

There is still argument as to whether or not a special entity, "alveolar cell carcinoma," really does exist. This is due mainly to the fact that the existence of an epithelial alveolar lining in healthy men was denied by several investigators (208, 150, 151, 181). The reviewer believes that sufficient evidence for an epithelium alveolar lining is established by the investigations of Koelliker (141), Bargmann (9, 10), Cappel (32), Clara (35) and recently by Low and Sampaio (165) using the electron microscope. Furthermore, he expresses the opinion (88) that there are some morphological similarities between normal cuboidal alveolar cells and alveolar carcinoma cells when originating from a well-differentiated type, also called lung adenoma.

Interestingly, our own false positive results have occurred in cases with lung fibrosis showing increased cuboidal alveolar cell proliferation and increased variation in nuclear size. In some respects one is reminded of the difficult situation when trying to differentiate squamous metaplasia from squamous carcinoma by cytological means. The concept that mutated or altered cuboidal alveolar epithelium may be transferred into alveolar cell carcinoma is suggested by the following experimental and clinical findings.

1) Induction of pulmonary tumors in vivo by chemical agents produces alveolar cell-like tumors (81, 140).

2) Some clinical investigators feel that conditions with fibrosis and chronic hyper-proliferation of cuboidal alveolar cells have an increased tendency for 'atypical proliferation' and for alveolar cell carcinoma (14, 219, 293). Clinical considerations for such theories have become so strong that they were expressed by Muschenheim (186) in the Yearbook of Medicine. However, Williams (278) denies such a relationship between "alveolar metaplasia" and carcinoma.

According to the various opinions on the normal histology of the lung, there is certain confusion concerning the terminology of this tumor, ranging from lung adenoma over bronchogenic carcinoma (156) to alveolar cell carcinoma. Kahalu (130) gives a comprehensive survey on the variety of opinions expressed, including the questionable relationship to jagziekte disease.

From the clinical point of view, some facts should be mentioned which might encourage further investigations in the differentiation of alveolar cell carcinoma from common bronchogenic adenocarcinoma.

The well-differentiated form of alveolar cell carcinoma (lung adenomatosis) definitely seems to have a more benign course than adenocarcinoma. Delayed tendency to metastasize, long follow-up observations by x-ray (3) and good survival rates in localized lesions (228), where surgery was possible, prove this statement. According to Storey et al. (256), non-generalized unilateral disease in which surgery is still possible might occur in 42% of the cases (solitary nodular lesions in 26%, lobe consolidation in 16%). Additional information may be obtained from a paper of Look and Krückemeyer (159).

As a rule, cytodiagnosis by examination of sputa or bronchial secretions is the most promising diagnostic procedure. in alveolar cell carcinoma. Eight of our eleven cases were so diagnosed; Griffith et al. (87) reports positive cytology in 86%, and Storey et al. (256) have obtained the same impression by reviewing the literature and looking over their own series. Biopsy during bronchoscopy, however, offers only a poor chance for diagnosis. So far cytodiagnosis is most contributory in diagnosing alveolar cell carcinoma. However, my own experience shows that a differentiation by cytologic means between the polymorph type of alveolar cell carcinoma and bronchogenic adenocarcinoma is not possible. Only in instances when exfoliation of cells takes place, which are even in size and which show mucus production and a tendency for papilloma-like grouping might the suspicion of an underlying alveolar cell carcinoma raised by the cytologist. From the clinician's point of view this situation does not have too many disadvantages, since it is only the well-differentiated lung adenomatosis type of alveolar cell cancer which offers good therapeutical chances.

Microphotographs illustrating the cellular patterns of this tumor type have been published among others in the follow-papers: (88, 89, 198, 286, 289).

5. Cytologic Diagnosis of Sarcoma and of a Combination of Etiologically Different Diseases

Probably due to an extremely low tendency for exfoliation and due to the rare incidence of lung sarcoma, no reports have been published up to date on the cytologic diagnosis of lung sarcoma (to the reviewer's knowledge). By the courtesy of Dr. Woolner (Rochester) and Dr. Lopes Cardozo (Leiden), this reviewer had the opportunity of seeing the sputum smears of sarcomas of the lung. There were giant cells and some spindle-shaped cells. The cytoplasm was not sharply outlined. However, the reviewer does not feel that a specific diagnosis of sarcoma of the lung by means of single exfoliated cells is possible with the procedures available at the present time. The probability of diagnosing a malignant tumor which may later turn out to be of sarcomatous character will be extremely rare.

The combination of tuberculosis and carcinoma is well known and the value of cytodiagnosis in such cases was pointed out relatively early by Bergmann et al. (17). However, the first diagnosis of complicating or accompanying tuberculosis was almost always made previously by bacteriologic studies. It seems to happen, quite rarely, that diseases

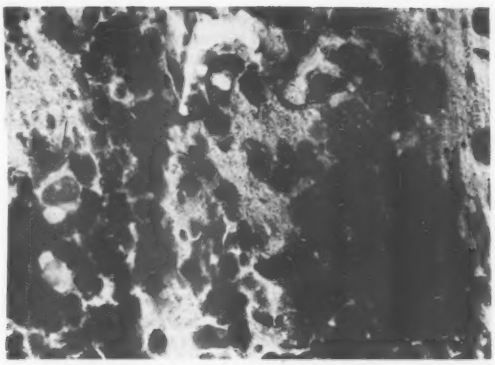


FIG 16. Sputum, Giemsa stain. On the right side a group of tumor cells; on the left side lymphocytes, caseous material and one epitheloid cell, showing a bean-shaped nucleus and a pale cytoplasm.

such as tuberculosis, or any other kind of granulomatous disease, in combination with a carcinoma, are both primarily detected by cytologic means. Recently we observed such a case which was later proved by histological examination of the surgical specimen (Fig. 16).

6. Cytodiagnosis as a Method for Mass Screening

Reagan and Schmidt (215) and others showed that cytodiagnosis is a valuable method for the detection of unknown and symptomless gynecological cancers. Similar studies in the detection of lung cancer turned out to be too expensive and inefficient in non-selected patient groups. Furthermore, the examination of one sputum specimen was too unreliable in small lesions.

Meanwhile, Papanicolaou and Irena Koprowska (192) were able to report a positive cytologic diagnosis in a case which showed no diagnostic radiologic changes at all. A few similar cases have been discussed during the cytologic meetings of the last year. Barach expressed at the second Workshop Conference on Lung Cancer Research (Harriman, 1959, Am. Cancer Soc.) the opinion that cytologic mass screening programs for lung cancer detection might be possible since the inhalation procedures have improved the quality of specimens significantly. The reviewer does not share this opinion because his own experience indicates that even after applying inhalation procedures, the single cytologic sputum examination is very unreliable in small lesions. The conclusion expressed by Day (41) in 1952 seems still true today: "Mass screening programs for lung cancer must depend primarily upon community photoroentgen surveys and routine fluoroscopy or chest roentgenograms in physicians' offices and in hospitals. Once this initial screening for silent lung tumors has been accomplished, cytological techniques provide one of the most effective methods of establishing the nature of the lesion." Before making this statement, Day discusses how more time-consuming cytodiagnosis is in sputum cytology than in gynecological cytology.

If done at all, I feel that a cytologic screening program should be restricted to selected population groups known to have a higher incidence of lung cancer.

At the present time cytodiagnosis is very valuable as a clinical method. It helps to detect or confirm lung cancer "earlier" than other methods. However, in most instances, it does not detect "early lung cancer," in the reviewer's opinion, since even small shadows on the chest-film indicate an advanced stage, having already caused destruction of the normal tissue. Shiller-Volkova (243) and Kohn et al. (143) have reported on such cases. Any optimism that we are detecting really early lung cancer in situ, is unjustified at the present when reviewing the overall survival rates after any kind of treatment.

The critical attitude of surgeons toward cytodiagnosis, for instance as expressed by Blacha (22) or Salzer et al. (230) might be mentioned only briefly. These authors and a number of other surgeons feel that repeated cytological examinations waste too much time in many cases and that exploratory thoracotomy has to be done anyway. Investigations now underway and experience of physicians indicate, however, that the use of exploratory thoracotomy is not as riskless and negligible in the prognosis of older patients as was thought previously. Complications and impaired cardiovascular function, as well as impaired pulmonary function, do not become apparent during surgery when the patient is saturated with oxygen but will arise later, usually so late that predominantly the physician is faced with the patient's post-surgical problems. Therefore, initially it seems worthwhile to make any attempt and to spend sufficient time in establishing a diagnosis by means of internal medicine.

No final conclusion seems possible at present in determining the future possibilities of the new methods of cancer detection in the peripheral blood in relation to the control of known bronchogenic cancer. A first study, especially concerned with lung cancer, has been performed by Colombo et al. (38).

In gynecology, reports have been published and discussions are going on as to whether or not it is possible to get some information by cytologic means on the sensitivity and response of a given tumor to radiation or other therapeutic procedures. No similar attempts have been made in relation to bronchogenic cancer, to the reviewer's knowledge.

B. CYTODIAGNOSIS OF ASPIRATED MATERIAL BY LUNG PUNCTURE

Monographs, especially dedicated to cytodiagnosis of aspirated lung material, have not been published yet. A comprehensive survey on the historical development of this diagnostic procedure will be obtained when studying the papers of Smetana (248), Woolf (283) and Grunze (88). Godlowski (78) and Grunze (88, 89) have studied and documented by microphotographs the morphologic patterns of nonmalignant disorders in aspirated material of the chest. Special reference is given to the variability of alveolar epithelium during its different stages of development.

Using purposely the term "aspirated material of the chest" indicates that not only lung tissue might be obtained when performing a "lung puncture." Cellular material of mediastinal organs, lymph nodes and the pleura may be a diagnostic constituent of the smear as well. Mostly this will happen when puncturing through the bronchi during bronchoscopy (24, 88, 89, 90). As outlined in the introduction to this article, such an expected or unexpected finding will be not only contributory to diagnosis but also provide some information on the topography of the lesion and its tissue matrix.

1. Methods for Obtaining Specimens by Needle Aspiration in Chest Diseases

Depending on the site of the lesion, specimens for cytological examination may be obtained by puncturing through the chest wall, through the bronchus during bronchoscopy (24, 185, 236, 237), through the esophagus during esophagoscopy (24), during thoracoscopy (6, 24, 89, 235) and transternally in thymic neoplasms (53).

Local anesthesia as described by Martin and Ellis (172), Schiepatti (236, 237) or Rosemond et al. (226, 227) is usually applied during such a diagnostic procedure. Recently general anesthesia in controlled artificial respiration by intubation based on a short acting barbiturate and curare-nitrogenoxide has been recommended by Brandt (24), Grunze (88, 89) and Mülly (185). In the experience of these authors fatalities and minor accidents are reduced significantly when applying this kind of anesthesia. Full control of the relaxed patients prevents not only air embolism, by neutralizing the negative pressure in the chest when stopping the artificial ventilation,

but also provides better diagnostic results (according to these authors).

A high rate of major and minor accidents during lung puncture and a spread of tumor cells with following colonization in the pleural cavity or the puncture channel have been, and still are, the main reasons for rejection of this diagnostic procedure.

The various possible accidents during lung puncture have been listed by Rosemond et al. (226), who published probably the largest series on this hazardous technique. In 272 needle aspirations of the lung done with local anesthesia, 20 wree complicated. Three patients died during the procedure, three others died after an interval and 14 showed pneumothorax, fever or other small discomfort. This valuable informative report mentions also the contra-indications for lung puncture, such as emphysema bullosum or status post-pneumonectomy.

Other reports on the possible hazards of lung puncture have been published by Gledhill (74) and Söderstrom (247), and a survey on the literature is given by Woolf (283) and Smetana (248).

Colonization of tumor cells in the puncture channel has been reported first by Reincke in 1870 (217). Although low in incidence, this complication has been repeatedly mentioned as one of the contra-indications for lung puncture. References may be found in articles of Smetana (248) and Grunze (88, 89). Mainly surgeons, who primarily were anxious to avoid procedures which interfered with the operability of a patient, were concerned with this problem (105).

Surveying the literature on diagnostic lung puncture from the very beginning, one is impressed by the fact that this technique was applied with almost complete safety in a large series of cases of lobar pneumonia for bacteriologic studies, experimentally (260) as well as clinically (234, 254, 283). Major and minor accidents and bacterial infection of the puncture channel occurred only to a negligible extent. In contrast to this favorable experience, the same procedure applied for tumor diagnosis was extremely hazardous and risky. Looking for the reasons for this striking difference, the reviewer and others noticed one main fact: The caliber of needles used for tumor diagnosis was always larger than that of needles used for bacteriological studies. Sometimes thick needles have purposely been used in order to obtain larger specimens for histological examination. This practice did not take into consideration the experiences of those who have done diagnostic lymph node punctures (88, 89, 93, 255) which proved that thin needle puncture is without risk and practically free of tumor spread, whereas ascites puncture with thick trocars frequently produces implantation metastasis even today. Furthermore, it is obvious that large caliber instruments will more easily cause lung lesions with following complications than would smaller needles. As a consequence of all these risks mentioned above, this reviewer favors the use of thin needles and of controlled artificial respiration in general anesthesia for lung puncture. He definitely shares the opinion of other authors that lung puncture should be applied only in cases where all other techniques have failed to establish a diagnosis.

In many instances, aspirated material from the chest contains so much blood that smears may be prepared in the same manner as peripheral blood smears. Small particles should be slightly squeezed. Other aspirates are so scant in material that the few small particles in the lumen of the needle have to be directly blown on a glass slide and carefully spread with a small instrument.

The Dudgeon and Patrick wet film technique, the Papanicolaou stain, the hematoxylin eosin stain, the Pappenheim or Giemsa stain and the phase contrast method have been applied on such preparations.

2. Value and Results of Needle Aspiration in Chest Diseases (Results Obtained by Histological Techniques Included)

Providing results even in lesions of the chest where no exfoliation of diagnostic cells is possible, seems to be one of the main advantages when comparing this technique with the various methods of exfoliative cytology. Lymph nodes, mediastinal and pleural tumors, lack of bronchial drainage or bronchial obstruction in bronchogenic and metastatic lung tumors and the tendency of some tumors not to exfoliate (benign tumors, sarcomas), as well as encapsulated inflammatory coin lesions, have been the most frequent reasons indicating needle aspiration. In our own series of 300 tumors of the chest (mediastinal, lymphatic, pleural and benign tumors included), 14% were diagnosed by needle aspiration after other methods had failed. Interestingly enough, lung puncture seems to be a method which often provides diagnostic results in rare pathologic conditions of the chest. Our series (88, 89, 90) contains, in addition to various systemic disorders of the lymphatic system, benign and malignant teratomas, cysts, lipomas, a thymoma and a chondroma. As a rule such diseases cannot be diagnosed by means of exfoliative cytology. The reviewer's critical remarks on the question, whether or not one can diagnose such rare conditions simply by exploratory thoracotomy, have already been mentioned in the foregoing section "cytology of sputum and bronchial secretions."

Reports on a few cases such as those published by Martin and Ellis (171) or Sharp (242) have been listed in a paper by Woolf (283). In larger series the following results were obtained. (See Table VIII).

TABLE VIII
RESULTS IN LUNG PUNCTURE

| | No. of patients | Positive results |
|---|---|---|
| Gledhill et al. (74):..... | 56 | 78 % |
| Rosemond and co-workers (226):... | 220 | 61 % |
| Schiepatti (236, 237):..... | 83 | 83.1% |

According to the reported positive results and disregarding the hazards of this diagnostic technique, lung puncture seems to be as efficient as sputum cytology. Further reference to the overall results of thin needle puncture is given in papers of Woolf (283), Smetana (248), Söderstrom (247), Grunze (88, 89) and Hambert et al. (97).

C. CYTODIAGNOSIS OF SEROUS EFFUSIONS

Although we are dealing in this article only with chest diseases, reference is also given to findings in ascitic fluid, since the morphology of the normal mesothelium of this cavity and its cytologic problems are very similar to those in thoracic diseases. Reviewing the literature, two difficulties are usually found which cause diagnostic errors from the very beginning:

1. How to obtain good specimens.
2. How to process these specimens.
3. How to differentiate mesothelial cells from malignant cells when taking into consideration the remarkable polymorphism of the former under various conditions.

Following Quensel's fundamental papers in 1928, surveying monographs and handbook articles on the subject have been published by Zadek (291, 292), Wihman (275) and Grunze (89).

1. Collection and Processing of Serous Fluids for Cytological Examination

Since the publications of Paul Ehrlich it has been recommended that one moves the patient into different positions before performing the puncture in order to suspend the cellular material, which tends to gravitate to the lowest point in body cavities. Fluids which instantly clot, for example in mesotheliomas (52), may be aspirated into syringes already containing small amounts of heparin (24, 88).

Although it is possible to process clotted material using the various cell block techniques, the results will not be as good as those obtained with non-clotted material. For this reason clotting should be prevented (276). The various methods for anticoagulation and many of the major and minor modifications for the cell block technique in the examination of fluids have been surveyed in Wihman's monograph. As opposed to Josephson (128), Mandlebaum (168) and others, Wihman spins down the concentrated exudate in a small sack of sausage skin floating in a centrifuge tube filled with water. Later the whole sack with the centrifuge sediment is fixed in 10% formalin, embedded in paraffin, and sectioned. Dietrich Mohr and myself, in the Cytology Laboratory, Chicago Lying-In Hospital, University of Chicago, used fingers from old India-rubber gloves which were perforated at different spots just prior to immersing them in formalin, with the same good results.

Wihman's method protects the fixed sediment from being partly destroyed when taken out of the centrifuge tube.

Usually the hematoxylin-eosin stain was applied as a routine method for staining cell block sections of sediments from serous fluids; however, special stains, applicable to formalin or alcohol-fixed material, also have been used when indicated.

From specimens containing too much peripheral blood, the diagnostic fraction of white cells may be separated according to a method inaugurated by Fawcett and Vallee (63) and critically evaluated by Elizabeth McGrew (178, 179). Jelly-like viscid material may be treated with hyaluronidase before centrifugation (108).

Small specimens or specimens poor in cellular content may be filtered according to the method of Kerrison and Chester (136). This procedure permits the entire sediment to be placed on a glass slide without loss of a particle and allows this sediment to be quantitatively examined after dissolving the filter membrane and staining the cellular materials.

Another technique for cytologic examination of serous fluids poor in cellular content was suggested by Sano (232), using cell culture. This method yielded positive results in tumor exudates when other methods failed. However, confirmation of results obtained with this minute technique has not been established sufficiently as yet, to my knowledge.

As a rule, the surface layer of the sediment contains the diagnostic white cells and the majority of the smears should be prepared from material of this area. In rare instances, however, sedimentation in three or more layers (tissue particles, mucus, fibrin) takes place and the bottom layer, containing adenocarcinoma cells with marked mucus production, may be most diagnostic (89).

Besides phase contrast microscopy (1), the Pappenheim and Giemsa stain (253) and the Papanicolaou stain (by the majority of authors) have been used as routine methods for native preparations of smears of sediments from serous fluids. In addition, Wittekind and Völker (280) recommended a combination of phase contrast and fluorescence microscopy as a screening procedure.

### 2. How to Differentiate Mesothelial Cells from Malignant Cells, Taking into Considerstion the Remarkable Polymorphism of the Former under Various Conditions

This problem has been of great importance in serous effusions from the beginning of cytology, since the majority of false positive diagnostic results were caused by misinterpretation of mesothelial cells.

Up until now no special staining procedures have been successful in providing definite differentiation in borderline cases. Even use of radioactive material (252) failed to improve this situation. Attempts recently made by Ceelen (33) with the PAS stain have not, to my knowledge, been confirmed yet.

A detailed knowledge of the different stages of development of mesothelial cells obtained by various staining methods (88, 89, 200, 252, 253) is helpful to a certain extent. Very informative in this respect is a histologic study by Sinapius and Vollhaber (245) on the normal morphological variation of mesothelial cells. Tables comparing characteristic properties of mesothelial cells with those of tumor cells are available (89, 200). Acinus-like organoid formations of mesothelial cells which cause confusion have been described (233). The cytomorphology and behavior of mesothelial cells in effusions were already mentioned in contributions which have been noted in the historical section of this review.

### 3. Cytodiagnostic Results on Serous Effffusions

The number of qualitative cellular reactions in serous effusions is limited according to the few varieties of cells lining or invading the pleural, pericardial or peritoneal cavities. As a result there is only one pathognomonic finding, that of tumors cells in carcinoma, sarcoma or malignant systemic disorders. In effusions induced by inflammations, congestions and mechanical means we are usually dealing with "constellations of cellular reaction." Their diagnostic value increases when correlated and compared with the clinical and bacteriological data. Some remarks on the classification of exudates and transudates will illustrate this situation.

A systematic classification of effusions based on the predominant cell type was first given by Widal and Ravaut (273, 274). Based on this grouping, similar attempts have been made recently (89, 276).

The following groups of effusions may be distinguished:

a) Predominantly mesothelial cells. (Cardiac and renal failure, metastatic tumors, consecutive sterile pleurisies in non-tuberculous lung diseases, small pulmonary infarctions.)
b) Predominantly lymphocytes. (Uncomplicated tuberculous effusions after having passed the initial stage; rarely in other chronic infections, renal effusions and tumors with marked reaction of the connective tissue and scar formation.)
c) Predominantly neutrophilic leukocytes. (Initial empyema, old effusions in residuals of old pneumothoraces, sterile exudates after pneumolysis or pneumonectomy).
d) Markedly increased eosinophilic leukocytes. (A nonspecific finding occurring in various diseases (241). According to Wihman (276) more frequent in allergic and rheumatic conditions, but also seen in infarctions, tumors or in such a benign condition as the small initial exudate after application of an artificial pneumothorax (281). From our own experience the majority of patients with eosinophilic pleurisy do not show symptoms of generalized allergy.)
e) Mixed neutrophilic-mesothelial pattern. (Sterile consecutive pleurisies in non-tuberculous infections with subpleural lesions, usually passing over into the neutrophilic form. Effusions in large pulmonary infarctions after the initial phase, usually passing over into the neutrophilic form after secondary infection.)
f) Hemorrhagic effusions. (Trauma, malignant tumors, acute forms of pleurisy in miliary tuberculosis.)
g) Predominantly tumor cells.
h) Neutrophils and debris only (empyema).

An instructive example, showing the importance of clinical data for the interpretation of cytologic findings in serous effusions, is furnished by the following remark of Wihman (276): "When a case with a prolonged course extending for more than three months, with an effusion of an exudate type, begins steadily to develop in the thoracic or abdominal cavity and, furthermore, when a distinct endothelosis without fibrin between the cells is ascertainable in this effusion, justification is obtained for the assumption of a tumor exudate, even though no specific tumor elements are to be found."

According to our experience this rule is very valuable in diagnosing early stages of metastatic pleurisy exhibiting, in

addition to abundant mesothelial cells, so few tumor elements that they are not detected. This rule is also valuable in cases failing to exfoliate tumor cells into the pleural cavity at all due to growth beneath the pleural surface and causing effusion only by lymphatic obstruction.

Based on the cytomorphological finding alone the result would be in such situations: "Abundant mesothelial cells, no tumor cells present (Class 1 or 2)." Taking into consideration the clinical and bacteriological data, the report may be formulated as follows: "Sterile effusion of exudate type with mesothelial cells. If congestion and mechanical reasons can be ruled out, suspicion for an underlying malignancy arises." Both formulations of diagnosis are correct. However, it is obvious which one is more contributory to diagnosis and therapy. It might be fair to state that consideration of clinical and bacteriological data in cytological specimens of serous effusions will significantly decrease false negative results.

Exceptionally tumor cells will be present in effusions of transudate type (low specific gravity) in patients suffering from protein deficiency (recent experience of the author).

Overall results on cytodiagnosis of malignant disorders in serous effusions have been published by Goldmann (79), Graham et al. (86), Honigmann (119), Perou and Littmann (200), Philipps and McDonald (204), Saphir (233) and others. Special reference to lymphomas is given in reports of Dawe et al. (40) and Glukhenkii and Bralibei (77). Both groups of investigators were unable to confirm the presence of characteristic cells, such as Sternberg-Reed cells, permitting a specific diagnosis of Hodgkin's disease. A recent report of Perou and Littman (200) and our own experience, however, shows that such a diagnosis is possible when using different staining methods. Perou and Littman have the impression "that the cytologist too often searches only for carcinoma cells," and the interpretation of the other types of cells present is neglected.

Favorable results in the diagnosis of metastatic cancer have been reported by Saphir (233), favoring the smear technique, however using both the cell block technique, which preserves better tumor structure (88, 200), and the smear method which provides better results in diagnosing single tumor cells or small size tumors (88). In 45 cases with evidence of carcinoma colonization on the pleura or peritoneum Saphir obtained 37 correct positive results, six doubtful results, and one false positive result. The average of correct positive results lies between 50–60% (86, 89). Determination of primary site and histologic type was found to be possible by Foot (68) in 50%.

Special difficulties have been reported in the diagnosis of oat cell carcinoma (253) and of mesothelioma (88, 200). The variation of morphology of mesothelial cells and histologic structures of mesotheliomas is extensive (173), beginning with "benign appearing mesothelial cells" similar to pulmonary infarction, going to spindle cell-like sarcoma structures (Lopes-Cardozo, personal communication) and ending with papilloma-like and adenocarcinoma-like groupings, as noted by Perou and Littman (200), Liebow (156), and also by this reviewer. Diagnosis is not difficult when polymorphic type cells are present. However, the diagnosis is difficult in cases with "benign features," since the most valuable criterion is lacking in such a situation, i.e., the finding of "strange looking cells" in addition to "normal mesothelial cells."

The possibilities of aspiration biopsy in cases with obliteration of the pleural space by tumor growth are mentioned in papers by Donohoe et al. (48) and others. Cytologic control of cytostatic therapy has been reported by Streicher (257) and Dova et al. (49). Moore et al. (182) have studied the influence of surgical procedures on the tumor cell content of serous fluids. No increase was noted after surgery.

## V. COMPARISON OF THE VALUE OF VARIOUS METHODS IN CYTODIAGNOSIS OF CHEST DISEASES

Returning to the introduction of this review and keeping in mind the question: "What is the overall usefulness of cytodiagnosis in chest diseases when applying all available methods?" one will find that the average positive correct results in tumor diagnosis range between 80 and 90%. Interestingly, this result has been obtained by different authors with different methods (Table VIII).

TABLE VIII

(Incomplete data and varying terminology do not permit direct comparison of results from author to author.)

| | | |
|---|---|---|
| Watson et al. (270): Diagnoses of 400 patients, 236 with bronchogenic cancer. | | |
| 112 patients | | sputum smear |
| 29 patients | | bronchial washing |
| 63 patiesnt | | biopsy |
| 85 patients | | aspiration biopsy |
| Rosemond et al. (227): Diagnoses of 328 patients with lung cancer. | | |
| 178 patients | (54.3%) | bronchoscopy and biopsy |
| 102 patients | (31.1%) | lung puncture |
| 18 patients | (5.5%) | exploratory thoracotomy |
| 20 patients | (6.1%) | lymph node biopsy |
| 5 patients | (1.5%) | bronchial secretion |
| 5 patien*s | (1.5%) | pleural effusion |
| Jennings and Shaw (126): Diagnoses of 64 patients with tumors of the lung. | | |
| | (37.1%) | sputum |
| | (16.3%) | bronchial biopsy |
| | (23.6%) | cytology and biopsy |
| | (4.5%) | diagnosis confirmed by other means |
| | (18.5%) | unconfirmed |
| Grunze and Brandt (90): Diagnoses of 300 patients with tumors of the chest. | | |
| | (9.66%) | sputum |
| | (20.66%) | bronchial smear, washing, curettage, or aspiration |
| | (34.33%) | bronchial biopsy |
| | (3.33%) | biopsy from other sites |
| | (8%) | perbronchial puncture |
| | (1.33%) | peresophageal puncture |
| | (3%) | transthoracic puncture |
| | (1.66%) | puncture during thoracoscopy |
| | (5%) | pleural effusion |
| | (4%) | exploratory thoracotomy |
| | (5%) | definitive diagnosis by clinical examination |
| | (4%) | undiagnosed |

Comparison of the above listed authors demonstrates that favorable results may be obtained by different methods, depending on local facilities and individual training. The optimal approach should be characterized by a maximum of correct comprehensive results and a minimum of risks and inconveniences for the patient.

## BIBLIOGRAPHY


1. VON ALBERTINI, A.: Cytologische Exsudatbefunde m. dem Phasenkontrastverfahren. Schweiz. Ztschr. Path. et Bact. **11**:701, 1946.
2. APPEL, M. and BRONK, T.: Tumor cells in bronchial secretions. Amer. J. Clin. Path. **19**:320, 1949.
3. ARANY, L. S.: Bronchiolar (alveolar cell) carcinoma. Am. Rev. Tbc. **78**:632, 1958.
4. ASCHOFF, C., KUSTER, E. and SCHMIDT, J. W.: Hundert Jahre Zellforschung. Berlin, 1938, Borntraeger.
5. AUERBACH, O., GEVE, J., FORMAN, J., PETRICK, T., SMOLIN, H., MUEHSAM, G., KASSOUNY, D., and STOUT, A.: Changes in the bronchial epithelium in relation to smoking and cancer of the lung; a report of progress. New Engl. J. Med. **256**:97, 1957.

6. AUERSBACH K., GRUNZE, H. and TRAUTMANN, F.: Cytologische Diagnostik unklarer isolierter Hilusveraenderungen durch gezielte Punktion. Tuberkulosearzt **7**:123, 1953.
7. BAMFORTH, J.: The examination of the sputum and pleural fluid in the diagnosis of malignant diseases of the lung. Thorax, London **1**:118, 1946.
8. BARBIER, F.: Over de cytologische Kankerdiagnostiek in de inwendige ziekten. Belg. Tijdschrift voor Geneeskunde **14**:655, 1958.
9. BARGMANN, W.: Zur vergleichenden Histologie der Lungenalveole. Z. Zellforschung. **23**:335, 1935/36.
10. BARGMANN, W.: "Die Lungenalveole." Handbuch der mikroskopischen anatomie des Menschen. Berlin, 1936, Springer.
11. BARRET, N. R.: Examination of the sputum for malignant cells and particles of malignant growth. J. Thorac. Surg. **8**:169, 1938.
12. BEALE, L. S.: Examination of sputum from a case of cancer of the pharynx and the adjacent parts. Arch. of Med. **2**:44, 1860.
13. BEALE, L. S.: The Microscope in Medicine. London, 1879, J. & A. Churchill.
14. BEAVER, D. L. and SHAPIRO, J. L.: A consideration of chronic parenchymal inflammation and alveolar cell carcinoma with regard to a possible etiologic relationship. Am. J. Med. **21**:879, 1956.
15. BECKER, W. H. and KNOTHE, W.: Intraoperative Fehldiagnosen: Bronchialkarzinom. Thoraxchirugie **6**:235, 1958.
16. BENCE, A. E.: El Lavado Bronquial en le diagnostico precoz del cancer de pulmon. Sémana Med. **47**:836, 1938.
17. BERGMANN, M., SHATZ, B. A. and FLANCE, J. J.: Apical pulmonary carcinoma and tuberculosis. The value of sputum cell study in the differential diagnosis. J. Amer. Med. Assoc. **138**:798, 1948.
18. BETSCHART, E.: Ueber die Diagnose maligner Lungentumoren aus dem Sputum. Arch. Path. Anat. Physiol. u. Klin. Med. **142**:86, 1895.
19. BEZAÇNON, F. and DE JONG, S. J.: Traité de l'examen des Crachats. Paris, 1912, Masson.
20. BICKERMAN, H. A., SPROUL, E. E. and BARACH, A. L.: An aerosol method of producing bronchial secretions in human subjects. Dis. of the Chest. **33**:377, 1958.
21. BITSCHIN, P.: Der Wert der cytologischen Zelldiagnostik zur Fruehdiagnose des Lungen-und Bronchialcarcarzinoms. Schweiz. Med. Wschr. **6**:128, 1953.
22. BLACHA, H. G.: Erfassung des Bronchialkarzinoms. Muenchener Med. Wschr. **100**:534, 1958.
23. BOLLACK, C.: Diagnostic des tumeurs par la technique des frottis. Lyon, 1952, Pidancet.
24. BRANDT, H. J.: "Beitraege zur Technik der Materialgewinnung fuer die cytologische Untersuchung bei Thoraxkrankheiten." Klinische Zytologie der Thoraxkrankheiten (H. Grunze). Stuttgart, 1955, Ferdinand Enke.
25. BROCA, P.: De la propagation de l'inflammation. Quelques propositions sur les tumeurs, dites cancéreuses. These, Paris, 1849.
26. BROCARD, H., and LAVERGNE, G. H.: Le récherche des cellules neoplastiques dans l'expectoration au cours des cancers bronchiques. Presse Med. **60**:730, 1952.
27. BRUNNER, A.: Der Lungenkrebs. Schweiz. Med. Wochenschr. **81**:653, 1951.
28. BRUNNER, A.: Vortrag auf dem Krebskongr. in St. Gallen 1950. Schweiz. Med. Wschr. **81**:653, 1951.
29. BUCHER, U.: Die Bedeutung gezielter Sputumuntersuchungen in Diagnostik und Therapie der Chron. Bronchitis. Schweiz. Med. Wochschr. **87**:1487, 1957.
30. BUFFMIRE, D. K. and MCDONALD, J. R.: Factors responsible for the failure of cytologic examination in cases of bronchiogenic carcinoma. S. Clin. North America **31**:1191, 1951.
31. CARABELLO, A.: Cytologic patterns in bronchopulmonary disease. Am. Rev. Tbc. **77**:22, 1958.
32. CAPPEL, D. F.: Observations on the origin of the mononuclear phagocytes of the lung. J. of Path. **26**:430, 1923.
33. CEELEN, A.: The PAS reaction as an aid in the interpretation of pleural and abdominal cytology. Transact. Inter-Society Cytology Council. 1957.
34. CHIPPS, H. D. and KRAUL, L. H.: Cytologic alterations in pulmonary tuberculosis which simulate carcinoma. Can. Res. **10**:210, 1950.
35. CLARA, M.: Vergleichende Histobiologie des Nierenglomerulus und der Lungenalveole. Frankfurter Zeitschr. f. Pathologie und mikroskop. Anatomie **40/41**-147, 1936–37.
36. CLERF, L. H. and HERBUT, P. A.: Diagnosis of bronchogenic carcinoma by examination of bronchial secretions. Ann. of Otol. **55**:646, 1946.
37. CLERF, L. H. and HERBUT, P. A.: The value of cytological diagnosis of pulmonary malignancy. Amer. Rev. Tbc. **61**:60, 1950.
38. COLOMBO, C., MAGGI, C. and ROLFO, F.: The presence of tumor cells in the peripheral arterial blood in patients with bronchial cancer. Minerva. Med. **49/17**: 725, 1958.
39. CROSS, K. R., CORCORAN, T. E., COOPER, T. J., and Landis, S. N.: Broncial carcinoma. Arch. of Path. **19**: 48, 1949.
40. DAWE, C. J., WOLLNER, L. B., PARKHILL, E. M. and MCDONALD, J. R.: Cytologic studies of sputum, secretions and serous fluids in malignant lymphoma. Amer. J. Clin. Path., **25**:480, 1955.
41. DAY, E.: Cytological techniques in screening uterine and lung cancer. Bulletin in Prog. in Cancer Res. (Am. Cancer Society) **2**:57, 1952.
42. DELARUE, M. M., PAILLUS, BERGERON-BLONDEL and GIACOBI: Précisions techniques sur le diagnostic cytologique des tumeurs bronchiques. Presse méd. **56(II)**: 580, 1948.
43. DELARUE, J. and ORÇEL, L.: Étude des resultats obtenus par les techniques cytologiques et des principales causes d'erreurs. Semaine Hop. Nr. **28**:1026, 1952.
44. DIGGS, C. W.: Use of Wright's stain in diagnosis of malignant cells in bronchial aspiration. Am. J. Clin. Path. **18**:293, 1948.
45. DIJKSTRA, B. K. S.: De Diagnostiek van Bronchuscarcinoom. Nederl. Tijdschr. Geneesk. **95**:3627, 1951.
46. DONALDSON, R.: The practicable application of the microscope to the diagnosis of cancer. Amer. J. Med. Sci. **25**:43, 1853.
47. DONNÉ, A.: Cours de microscopie complémentaire des études medicales. Paris, 1845, Baillière et Fils.
48. DONOHOE, R. F., KATZ, F. A. C. P. and MATTHEWS, M. J.: Pleural biopsy as an aid in the etiologic diagnosis of pleural effusions: review of the literature and report of 132 biopsies. Ann. of Int. Med. **48**:1588, 1957.
49. DOVA, E., MARINOM, U. and AVANZINI, S.: Modificazioni Citologiche Indotte d'all' Au 198 sull' Ascite Neoplastica. Tumori **43**:328, 1957.
50. DUDGEON, L. S. and PATRICK, C. V. A.: A new method for the rapid microscopical diagnosis of tumours with an account of 200 cases so examined. Brit. J. Surgery, **15**:250, 1927/28.
51. DUDGEON, L. S. and WRIGLEY, C. H.: On the demonstration of particles of malignant growth in the sputum by means of the wet-film method. J. Laryng. and Otol. **50**:752, 1935.
52. DVOSKIN, S.: Mesothelioma of the peritonaeum. Ann. Int. Med. **40**:809, 1954.
53. EFFLER, B. B. and McCormack, J. J.: Thymic neoplasms. J. Thor. Surg. **31**:60, 1956.
54. EFSKIND, L. and WEXELS, P.: Hodgkin's disease of the lung with cavitation. J. Thorac. Surg. **23**:377, 1952.
55. EHRLICH, P.: Beitraege zur Aetiologie und Histologie pleuritischer Erguesse. Charité-Ann. **7**:199, 1882.
56. EHRLICH, P.: Ueber die Methylenblaureaktion der lebenden Nervenendigungen. Biolog. Zentralblatt **6**: 624, 1886.

57. Ehrlich, P.: Ueber Neutralrot. Zeitschrift fuer wissenschaftl. Mikroskopie **11**:250, 1894.

58. Ehrlich, P.: For References in English: Collected papers of Paul Ehrlich. London and New York, 1956, Pergamon Press.

59. Escher, F. and Strupler, W.: Die Bedeutung der cytologischen Differenzierung der Bronchialsekrete fuer die Fruehdiagnose der Lungenkrebse. Bern, 1950, Praxis.

60. Farber, J. E., Carpenter, R. and Pellicano, V.: Lipoid pheumonia diagnosis by aspiration biopsy. Amer. Rev. Tbc. **45**:453, 1942.

61. Farber, S. M., Rosenthal, M., Alston, E. F., Benioff, M. A. and McGrath, K.: Cytologic Diagnosis of Lung Cancer. Springfield, 1950, C. C. Thomas.

62. Farber, S. M. et al.: The mucolytic and digestive action of trypsin in the preparation of sputum for cytologic study. Science (Lancaster, Pa.) **117**:687, 1953.

63. Fawcett, D. W. and Vallee, B. L.: Some new approaches to the cytologic diagnosis of cancer from serous fluids. Bulletin of the New England Medical Center. **12**:224, 1950.

64. Florentine, P., Pierson, B. and Grilliat, J. P.: Diagnostic Cytologique précose du cancer bronchopulmonaire. Strasbourg Med. 416, 1952.

65. Fontaine, R., Klein, M., Forster, E., Chardon, V. and Bollack, C.: Resultat des examens cytologiques dans le diagnostic des tumeurs du poumon. Strasbourg Med. **3**:419, 1952.

66. Foot, C. N.: The identification of tumor cells in sediments of serous effusions. Amer. J. Path. **13**:1, 1937.

67. Foot, C. N.: The identification of types of pulmonary cancer in cytologic smerars. Amer. J. Path. **28**:963, 1952.

68. Foot, C. N.: The identification of types and primary sites of metastatic tumors from exfoliated cells in serous fluids. Amer. J. Path. **30**:661, 1954.

69. Foot, C. N.: Critical analysis of smears from 1000 persons. Am. J. of Clin. Path. **25**:223, 1955.

70. Foot, C. N.: Cytologic diagnosis in suspected pulmonary cancer. Am. J. of Clin. Path. **25**:223, 1955.

71a Friedlander, C.: The Use of the Microscope in Clinical and Pathologic Examinations. New York, 1885, D. Appleton.

71b. Friedlander, C.: Microscopische Technik. Berlin, 1883, T. Fischer.

72. Frühling, L. and Wachenheim, J. P.: Diagnostic histopathologique du cancer pulmonaire par examens des moules bronchiques expectorées. Strasbourg med. 397, 1952.

73. Garret, M., Koprowska, T., Lions, H. A., Caro, C. G. and Eli, M.: The efficiency of the cytologic method in the detection of bronchogenic carcinoma, preliminary report. New York State J. Med. **58**:2041, 1958.

74. Gledhill, E. Y., Spriggs, J. B. and Binford, C. H.: Needle aspiration in the diagnosis of lung carcinoma. Amer. J. Clin. Path. **19**:235, 1949.

75. Gloyne, S. R.: The cytology of sputum. Tubercle **18**: 292, 1936/37.

76. Gloyne, S. R.: The cytology of sputum. Tbc. Abstr. 12, V., 1939.

77. Glukhenkii, T. T. and Bralibei, B. M.: The pleural fluid in lymphogranulomatosis. Ref. from the Russ. Vrach. Delo **3**:251, 1957 in Abstr. of Sov. Medicine. Expert. Medica (Amsterdam) **3**:30, 1949.

78. Godlowski, Z. Z.: Cellular analysis of the aspiration of the lung biopsy from normal and some pathologic conditions. J. Clin. Path. **2**:49, 1959.

79. Goldmann, A.: Cytology of serous effusions with special reference to tumor cells. Arch. Surg. **19**:1672, 1929.

80. Gowar, F. J. S.: Carcinoma of the lung: the value of sputum examination in daignosis. Brit. J. Surg. **39**:193, 1943.

81. Grady, H. G. and Stewart, H. L.: Histogenesis of induced pulmonary tumors in strain A. mice. Am. J. Path. **16**:417, 1940.

82. Graham, R. M.: The Cytologic Diagnosis of Cancer by the Staff of the Vincent Memorial Laboratory, Boston (Mass.). Philadelphia and London, 1950, Saunders.

83. Graham, R. M.: Diagnosis of cancer of internal organs by papanicolaou technique. Advances in Internal Medicine **6**:59, 1954.

84. Graham, R. M.: Reliability of cytodiagnostic methods in cancer detection. New York J. of Med. **58**:1265, 1958.

85. Graham, R. M.: Symposium on spindle cells. Acta Cytologica **2**:264, 1958.

86. Graham, G. G., McDonald, J. R., Clagett, O. T. and Schmidt, H. W.: Examination of pleural fluid for carcinoma cells. J. Thor. Surg. **25**:366, 1953.

87. Griffith, E., McDonald, J. R. and Clagett, O. T.: Alveolar-cell tumors of the lung. J. Thor. Surg. **25**:949, 1950.

88. Grunze, H.: Klinische Zytologie der Thoraxkrankheiten. Stuttgart, 1955, Ferdinand Enke.

89. Grunze H.: "Cytologie." Handbuch d. Inn. Med. Bd. 4, Teil 1. Berlin, 1956, Springer.

90. Grunze, H.: "Cytologie von Bronchien, Lunge und Mediastinum." Internat. Symposium ueber Klinische Cytodiagnostik in Erlangen. Stuttgart, 1957, Georg Thieme.

91. Grunze, H.: Discussion on staining procedures. Acta Cytologica **2**:468, 1958.

92. Grunze, H.: Symposium on spindle cells. Acta Cytologica **2**:264, 1958.

93. Guthrie, C. G.: Gland puncture as a disgnostic measure. Bull. Johns Hopkins Hosp. **32**:266, 1921.

94. Guttmann, D. and Smidt, H.: Uber Vorkommen und Bedeutung der Lungen alveolarepithelien in den Sputis. Zeitschr. Klin. Med. **32**:247, 1897.

95. von Haam, E.: Symposium on spindle cells. Acta Cytologica **2**:268, 1958.

96. O'Hagan-Ward: Exfoliative cytology in the diagnosis of cancer. S. Afr. Med. J. **32**:393, 1958.

97. Hambert, H., Thuilliez, A. G., Tserrenis and Foussier, B.: Le prélèvment a l'aiguille comme moyen diagnostic des tumeurs intrathoraciques. J. Franc. Med. et Chir. Thor. **4**:262, 1950.

98. Hampeln, P.: Ueber einen Fall von primaerem Lungen-Pleura-Carzinoom. St. Petersburger Med. Wschr. **17**: 137, 1887.

99. Hampeln, P.: Ueber den Auswurf beim Lungencarcinom. Zeitschr. f. Klin. Med. **32**:247, 1897.

100. Hampeln, P.: Zur Symptomatologie und Diagnose der Primaeren Malignen Lungentumoren. Mitt. Grenzgeb. Med. u. Chir. **31**:672, 1919.

101. Hampson, F.: Exfoliative cytology in the diagnosis of lung cancer: evaluation of one laboratory's results. Brit. M. J. **2**:1461, 1956.

102. Hampson, F.: Discussion on a report of Hjelt: cellular changes in bronchogenic carcinoma. Acta Unio. Internat. Contra Cancrum (Bruxelles) **14**:496, 1958.

103. Hartmann, P.: Die Cytologie des Bronchialsekretes. Stuttgart, 1955, Georg Thieme.

104. Hartmann, P., Greven, H. and Drewes, J.: Die Cytodiagnostik des Bronchialcarcinoms. Zschr. Laryng. **32**:577, 1953.

105. Hasche, E.: Zur Differentialdiagnostik des Bronchialkarzinoms. Bruns Beitr. **185**:129, 1952.

106. Hayek, H. V.: Die menschliche Lunge. Berlin, 1953, Spinger.

107. Heckner, F.: Zytologische Bronchialdiagnostik. Dtsch Med. Wschr. **77**:537, 1952.

108. Heckner, F. and Goeltner, E.: Zur Zytodiagnostik von Exsudaten. Erfahrungen mit Zusatz von Hyaluronidase. Dtsch. Med. Wschr. 1693, 1954.

109. HELLWIG, C. A.: Lung cancer; Value of cytological studies of bronchial secretions. J. of the Kansas Med. Soc. **59**:389, 1958.

110. HENGSTMANN, H. and WITTEKIND, D.: Erfahrungen in der cytologischen Tumordiagnostik mit der Methode nach Papanicolaou. Med. Klin. **45**:463, 1950.

111. HENGSTMANN, H.: Die Zytodiagnostik des Bronchialkarzinoms mit Hilfe der gezielten Absaugung. Ztschr. Klin. Med. **150**:183, 1953.

112. HERBUT, P. A. and CLERF, H. L.: Bronchogenic carcinoma: diagnosis by cytologic study of bronchosopically removed secretions. J. Amer. Med. Assoc. **130**:1006, 1946.

113. HERBUT, P. A.: Correlation of cytological with pathological findings in tumors of the lung. Proc. Symposium on Exfoliative Cytology 1951. (New York: American Cancer Society, 1953, p. 50).

114. HILDING, A. C.: Summary of some known facts concerning the common cold. Transactions of the Amer. Laryngol. Assoc. **66**:87, 1944.

115. HJELT, L.: Cellular changes in bronchogenic carcinoma. Sputum studies. Acta Unio. Internat. Contra Cancrum (Bruxelles) **14**:494, 1958.

116. HJELT, L.: A cytologic diagnosis of pulmonary cancer. Acta Path. et Microbiol. Scand. Supp. **93**:128, 1952.

117. HOESSLIN, H. V.: Das Sputum. Berlin, 1926, Springer.

118. HOFFMANN, F. A.: Die Bedeutung der Herzfehlerzellen. Arch. Klin. Med. **45**:252, 1889.

119. HONIGMANN, A. H.: The significance of tumor cells in serous effusions. J. Surg. etc. **81**:295, 1945.

120. HUGHES, A.: A History of Cytology. London and New York, 1959, Abelard-Schuman.

121. HUIZINGA, E.: Le cancer des bronches. Acta Otol. etc. Belg. **5**:337, 1951.

122. IVERSEN, JOHAN: *a*) Cytologik diagnostik of maligne lungesygdomme. Nord. Med. **49**:671, 1953; *b*) Falshe positive cytologisk. lunge diagnostik. Nord. Med. **49**: 674, 1953; *c*) Cytologisk lunge disgnostik. Nord. Med. **49**:676, 1953.

123. JACKSON, E., BERTOLI, F. and ACKERMANN, L. V.: Exfoliative cytology. J. Thor. Surg. **21**:7, 1951.

124. JAMPOLIS, R. W., MCDONALD, J. R. and CLAGETT, O. T.: Mineral oil granuloma of the lung. Surg. etc. **97**:105, 1953.

125. JANES, R. M.: Lipoidpneumonia simulating bronchogenic carcinoma. J. Thor. Surg. **16**:451, 1947.

126. JENNINGS, R. C. and SHAW, K. M.: The value of cytological examination of the sputum in the diagnosis of the bronchus. Thorax, **8**:288, 1953.

127. JOHNSON, W. D., KOSS, L. G. and PAPANICOLAOU, G. N.: Cytology of esophageal washings. Cancer **8**:591, 1955.

128. JOSEPHSON, A.: Nachweis von Geschwulstzellen in Exudaten, Harn und Lymphdruesen. Z. Klin. Med. **82**: 331, 1916.

129. KAHLAU, G.: Die praktische Bedeutung der zytologischen Untersuchung des Bronchialskretes. D.M.W. **14**:535, 1958.

130. KAHLAU, G.: Der Lungenkrebs. Erg. Path. **37**:258, 1954.

131. KAHLAU, G.: Ueber zytologische Untersuchungen von Expectoraten und Punktionsfluessigkeiten mittels der Methode von L. Silverstope. Klin. Wschr. 574, 1950.

132. KARP, H.: Cytodiagnostik maligner Tumoren bei Punktaten und Sekreten. Ztschr. f. Krebsforschg. **36**: 579, 1932.

133. KAWECKA, M.: Cytodiagnostika raka. Warsawa, 1956, Zaklad Wydan Lekarskirch.

134. KAWECKA, M.: Cytological evaluation of sputum in patients with bronchiectasis and the possibilities of erroneous diagnosis of cancer. Acta Unio. Internat. Contra Cancrum **15**:469, 1959.

135. KAWECKA, M.: Le cytodisgnostic du cancer bronchopulmonaire. Bull. de l'Assoc. Francaise pour l'étude du Cancer **45**:250, 1958.

136. KERRISON, J. and CHESTER, C. L.: A filter membrane technique for cytological study of exfoliated cells in body fluids. Cancer (U.S.) **12**:278, 1959.

137. KING, L. S.: Atypical proliferation of bronchiolar epithelium. A.M.A. Arch. of Path. **58**:59, 1954.

138. KJAER, T., DREYER, V. and HANSEN, J. L.: Clinical experiences concerning tumor cells in the sputum. Acta med. scand. (Stockh.) Suppl. **234**:177, 1949.

139. KLEMM-HARVEY, J. A. and HOOKER, J. W.: Cytology of bronchial aspirates in early diagnosis of bronchogenic carcinoma. Am. J. Clin, Path. **21**:550, 1951.

140. KOECKE, H. U.: Untersuchungen zur Empfindlichkeit der Alveolarepithelzellen in der Maeuselunge gegen cancerogene Stoffe (Methylcholanthren). Zeitschrift fuer Zellforschung und mikroskop. Anatomie **47**:331, 1958.

141. KOELLIKER, A.: Epithel der menschlichen Lungenalveolen. Sitzungsbericht physik.-med. Ges. Wuerzburg, 1880.

142. KONEIGER, H.: Die cytologische Untersuchungsmethode, Jena, 1908, Gustav Fischer.

143. KOHN, G., HORNSTEIN, L. and HIRSCH, W.: Routine examination of secretions and exudates for cancer cells by Papanicolaou method of exfoliative cytology in a medical laboratory. Harefuah J. of the Med. Assoc. of Israel **54**:9, 1958.

144. KOLYADITSKAJA, E. A.: Cytologic examination of the sputum in cancer of the lung.. Ref. from the Russ. (Klin. Med,) Moskow) 65 (1957) in Abstr. of Sov. Med., Exerpt Med. (Amsterdam) **3**:276, 1959.

145. KOSS, L. G. and RICHARDSON, H. L.: Some pitfalls of cytological diagnosis of lung cancer. Cancer **8**:5, 1955.

146. KOSS, L. G.: Cellular changes simulating bronchogenic carcinoma. Acta Unio. Internat. Contra Cancrum **14**: 501, 1958.

147. KOSS, L. G.: Symposium on spindle cells. Acta Cytologica **2**:268, 1958.

148. KRÖNIG, G.: Diagnostischer Beitrag zur Herz- und Lungenpathologie. Berlin, Klin. Wschr. 1887, p. 96.

149. KUCSKO, L. and PORTELE, K.: Ueber den Nachweis von Tumorzellen im Sputum. Krebsarzt **4**:183, 1949.

150. LANG, F. J.: Ueber Gewebskulturen der Lunge. Ein Beitrag zur Histologie des respiratorischen Epithels und zur Histogenese der Alveolar-phagozyten. Arch. exper. Zellforsch. **2**:13, 1926.

151. LANG, F. J.: Ueber die Alveolarphagocyten der Lunge. Virchows Arch. **275**:105, 1929.

152. LEBERT, M.: Traité practique des maladies cancéreuses et des affections curable confondues avec le cancer. Paris, 1851, J. B. Baillière.

153. LEYDEN, T.: Uber infektiöse Pneumonie. Dtsch. Med. Wschr. **9**:52, 1883.

154. LIEBMANN, E.: Ueber das Verhalten des Auswurfes bei der nekrotischen Influenzapneumonie. Schweiz. Med. Wochenschr. 727, 1920.

155. LIEBMANN, E.: Untersuchungen ueber die Morphologie des Auswurfes bei Lungentuberkulose. Z. Tbk. **32**:341, 1920.

156. LIEBOW, A. A.: Tumors of the lower respiratory tract. Washington, D.C.Armed Forces Institute of Pathology, Section V: Fasicle **17**:189, 1952.

157. LIEBOW, A. A., LINDSKOG, G. E. and BLOOMER, W. E.: Cytological studies of sputum and bronchial secretions in the diagnosis of cancer of the lung. Cancer (N.Y.) **1**:223, 1948.

158. LINK, R.: Ueber den Wert der zytologischen Untersuchung des Bronchialsekretes. Arch. Ohr. -u.s.w. Heilkunde u.Z. Hals -u.s.w. Heilkunde **161**:268, 1952.

159. LOOK, K. H. and KRÜCKEMEYER, K.: Klinische und morphologische Untersuchungen an elf Alveolar-Zell-Karzinomen. Ärtztl. Wschr. **13**:225, 1958.

160. Lopes-Cardozo, P.: Advantages and disadvantages of the May-Gruenwald Giemsa procedure in exfoliative cytology. Acta Cytologica **2**:284, 1958.

161. Lopes-Cardozo, P.: Het Herkennen van Gezwelzellen in volgens May-Gruenwald Giemsa Dekleurde Uitstrijkpraeparaten. Nederl. Tijd.-schr. Geneesk. **94**:1635, 1950.

162. Lopes-Cardozo, P. and Janssen, C. A.: De clinische Cytologie van Sputum en bronchuscurettement. Ned. Tijdschr. Geneesk. **94**:2318, 1950.

163. Lopes-Cardozo, P.: Clinical Cytology. Leiden 1954,

164. Losner, S., Volk, B. W., Slade, W. R., Nathanson, L. and Jacobi, H.: Diagnosis of lipoid pneumonia by examination of sputum. Amer. J. Clin. Path. **20**:539, 1950.

165. Low, N. L. and Sampaio, M. M.: The pulmonary alveolar epithelium as an entodermal derivative. Anat. Records (U.S.) **127**:51, 1957.

166. Lücke, A. and Klebs, E.: Beitrag zur Ovariotomie und Zur Kenntniss der Abdominalgeschquelste. Arch. Path. Anat. **41**:1–14, 1867.

167. Lüdin, H.: Tumorzellnachweis in Organpunktaten mit dem Phasenkontrastverfahren. Schweiz. Med. Wschr. 710, 1948.

168. Mandlebaum, F. S.: The diagnosis of malignant tumors by paraffin sections of centrifuged exudates. J. Lab. and Clin. Med. **2**:580, 1917.

169. Maroun, T.: La place du cyto-diagnostic dans le diagnostic précoce du cancer pulmonaire. J. Med. Libanais, Beirut **10**:6, 1957.

170. Martin, H. E. and Ellis, E. B.: Aspiration biopsy. Surg. Gynec. and Obstet. **59**:578, 1934.

171. Martin, H. E., and Ellis, E. B.: Biopsy by needle puncture and aspiration. Am. Surg. **92**:169, 1930.

172. Martin, H. E. and Ellis, E. B.: Biopsy by needle puncture and aspiration biopsy. Amer. J. Roentgenol. **35**:245, 1936.

173. Masenti, E. and Gullina, D.: Anstomical and clinical considerations in 18 cases of primary tumors of the pleura. Chir. Torac. **10**/**5**:375, 1957.

174. Matthews, W. H.: The examination of sputum for tumor cells. Canada Med. Assoc. **58**:236, 1948.

175. McBurney, R. P., Kirklin, J. W. and Woolner, L. B.: Metastasing bronchial adenomas. Surg. etc. 482, 1953.

176. McDonald, J. R. and Woolner, L. B.: Malignant tumors of the lung; their diagnosis by cytologic examination of sputum and bronchial secretions. Minnesota Med. **32**:1186, 1949.

177. McDonald, J. R.: Exfoliative, cytology in genitourinary and pulmonary diseases. Am. J. Clin. Path. **24**:684, 1954.

178. McGrew, E. A.: Concentration of cells from body fluids for cytologic study. Am. J. of Clin. Path. **24**:9, 1954.

179. McGrew, E. A. and Nanos, S. T.: Factors influencing the isolation of malignant cells by floatation. Trans. Inter-Soc. Cytology Coun. 1957.

180. McKay, D. G., Ware, P. F., Atwood, D. A. and Harken, D. E.: The diagnosis of bronchogenic carcinoma by smears of bronchoscopic aspirations. Cancer (N.Y.) **1**:208, 1948.

181. Miller, W. S.: The Lung. Springfield, Ill., 1950, C. Thomas.

182. Moore, G. E., Sandberg, A. A., Burke, E. M., Johnson, R. T. and Katz, A. D.: Tumor Cells in the Blood and Body Cavity Associated with Malignancy of the Lung and Gastrointestinal Tract. Surg. Forum Vol. VIII, 1958.

183. Mueller, Johannes: Ueber den feineren Bau und die Formen der Krankhaften Geschwuelste. Berlin, 1838, Reimers.

184. Mulder, J.: Bacteriology of chronic bronchitis. Proc. Roy. Soc. Med. **49**:773, 1956.

185. Müller, K.: Persoenliche Mitteilung zur Frage der perbronchialen Punktion. 1954.

186. Muschenheim, C.: "The Chest." Yearbook of Medicine, p. 113, 1957/58.

187. Nathanson, L., Fraenkel, D. and Jakobi, M.: Diagnosis of lipoid pneumonia by aspiration biopsy. Arch. Int. Med. **72**:672, 1943.

188. O'Keefe, J. J.: The cytologic diagnosis of primary bronchogenic carcinoma. Laryngoscope **60**:931, 1950.

189. Overholt, R. H. and Schmidt, J. G.: Survival in primary carcinoma of the lung. New Eng. J. Med. **240**:491, 1949.

190. Papanicolaou, G. N. and Traut, H. F.: Diagnosis of Uterine Cancer by the Vaginal Smear. New York, 1943, Commonwealth Fund.

191. Papanicolaou, C. N.: A new procedure for staining vaginal smears. Science **95**:438, 1942.

192. Papanicolaou, G. N. and Koprowoska, I.: Carcinoma in situ of the right lower bronchus. Cancer (N.Y.) **4**: 141, 1951.

193. Papanicolaou, G. N. and Cromwell, H. A.: Diagnosis of cancer of the lung by the cytologic method. Dis. Chest **15**:412, 1949.

194. Papanicolaou, G. N.: Historical development of cytology as a tool in clinical medicine and in cancer research. Acta Unio. Internat. Contre le Cancer **14**:249, 1958.

195. Papanicolaou, G. N.: The evolutionary dynamics and trends of exfoliative cytology. Texas Rep. Biol. and Med. Vol. 13 **4**:901, 1955.

196. Papanicolaou, G. N.: Degenerative changes in ciliated cells exfoliating from the bronchial epithelium as a cytologic criterion in the diagnosis of diseases of the chest. New York State J. Med. **56**:2647, 1956.

197. Papanicolaou, G. N.: Simple method for protecting fresh smears from drying and deterioration during mailing. J.A.M.A. **164**:1331, 1957.

198. Papanicolaou, G. N.: Atlas of Exfoliative Cytology. New York, 1956, Commonwealth Fund, Harvard Univ. Press.

199. Pappenheim, A.: Grundriss der Farbchemie zum Gebrauch bei mikroskopischen Arbeiten. Berlin, 1901, August Hirschwald.

200. Perou, M. L. and Littman, M. S.: Diagnostic study of serous effusions with emphasis on some unusual findings. Am. J. Clin. Path. **25**:467, 1955.

201. Peters, Hannah: Cytologic smears from the mouth. Am. J. Clin. Path. **29**:219, 1958.

202a. Pfaltz, C. R. and Probst, R.: Die normale und pathologische Cytologie des Bronchialsekretes und der Oesophagusschleimhaut. Arch. Ohr. -u.s.w. Heilkunde U. Z. Hals -usw. Heilkunde, **164**:255, 1953.

202b. Probst, R. and Pfaltz, C. R.: Uber cytologische Diagnostik in der Oto-Rhino-Laryngolie. Arch. Ohr -u.s.w. Heilk. und Zeitschr. Hals u.s.w. Heilk. **164**:197, 1953.

203a. Pharr, S. L., Farber, S. M. and King, E. B.: Cellular concentration of sputum for cytologic examination. Trans. Inter-Soc. Cytology Coun. 1957.

203b. Pharr, S. L.: Personal communication, 1959.

204. Phillips, S. K. and McDonald, J. R.: An evaluation of examinations performed on serous fluids Am. J. Med. Sci. **216**:121, 1948.

205. Philps, F. R.: Adenocarcinoma cells in the sputum. Brit. J. Cancer **8**:67, 1954.

206. Philps, F. R.: Appearances which may cause confusion in the diagnosis of bronchial carcinoma from sputum examination. Brit. J. Cancer **8**:422, 1954.

207. Pierson, B.: The cytodiagnosis of bronchial adenoma. 6th Ann. Inter-Society Cytology Council Meet. New York, 1958. (Report to be published.)

208. Policard, A.: Les nouvelles idees sur la disposition de la surface respiratoire pulmonaire. Presse Med. **2**:205, 1929.

209. QUENSEL, U.: Zur Frage der Cytodiagnostik der Erguesse seroeser Hoehlen. Acta Med. Scand. (Stockh.) **68**:427, 1928.

210. QUENSEL, U.: Cytologische Untersuchungen von Erguessen der Brust- und Bauchhoehlen mit besonderer Berucksichtigung der carzinomatoesen Exsudate. Acta Med. Scand. (Stockh.) **68**:458, 1928.

211. QUINCKE, H.: Ueber fetthaltige Transsudate. Dtsch. Arch. Klin. Med. **16**:121, 1875.

212. QUINCKE, H.: Uber die geformten Bestandteile von Transsudaten. Dtsch. Arch. Klin. Med. **30**:580, 1882.

213. RABE, R.: Zur Geschichte der Zellforschung im 19. Jahrhundert. Beitr. Path. Anat. u. zur Allg. Path. **119**:1, 1958.

214. RAVAUT, P.: Le diagnostic de la nature des épanchements sérofibrineux de la plêvre cytodiagnostic C. Naud. Edit. Paris 1901 and Gaz. Hop. 570, 1902.

215. REAGAN, J. W. and SCHMIDT, R. T.: Evaluation of cytologic technique in recognition of malignant uterine neoplasms. JAMA **145**:82, 1951.

216. REID, L.: Pathology of chronic bronchitis. Proc. Roc. Soc. Med. **49**:771, 1956.

217. REINCKE, J.: Zwei Faelle von Krebsimpfung in Punktionskanaelen bei carcinomatoeser Peritonitis. Virchows Arch. **51**:391, 1870.

218. REITTER, J.: Vergleichende cytohistologische Unter suchungen an boesartigen Tumoren unter besonderer Berueeksichtigung des Phasenkontrastverfahrens. Lagenbecks Arch. u. Dtsch. Z. Chir. **269**:329, 1951.

219. RICHARDS, R. L. and MILNE, J. A.: Cancer of the lung in progressive systematic sclerosis. Thorax **13**:328, 1958.

220. RICHARDSON, H. L., KOSS, L. G., and SIMON T. R.: An evaluation of the concomitant use of cytological and histological techniques in the recognition of cancer in exfoliated material from various sources. Cancer **8**: 948, 1955.

221. RICHARDSON, H. L., HUNTER, W. C., CONKLIN, W. S. and PETERSEN, A. B.: A cytologic-histologic study of bronchial secretions. Am. J. Clin. Path. **19**:323, 1949.

222. RIEDER, H.: Zur Diagnose der Neubildungen bei klinische-mikroskopischen Untersuchungen von Transsudaten. Dtsch. Arch. Klin. Med. **54**:544, 1895.

223. RIEGEL, R.: Phasenkontrastmikroskopische Bronchuscytologie. Zeitschr. fuer Klin. Med. **151**:446, 1954.

224. ROBINSON, C. L. and JACKSON, C. A.: Multiple primary cancer of the lung. J. of Thoracic Surg. **36**:166, 1958.

225. ROME, D. S.: The value of aerosol produced sputum as a screening technique for lung cancer. Acta Unio Internat. Contra Cancrum **15**:474, 1959.

226. ROSEMOND, G. P., BURNETT, W. E. and HALL, J. H.. Value and limitations of aspiration biopsy for lung lesions. Radiology **52**:506, 1949.

227. ROSEMOND, G. P., BURNETT, W. E. and HALL, J. H.: Diagnosis of carcinoma of the lung. J. Int. Coll. Surg. **14**:100, 1950.

228. ROSEMOND, G. P., BOUCOT, K. R. and ALGERTER, E.: Solitary pulmonary adenoma (focal pulmonary adenomatosis): A three year follow up after resection. J. Thor. Surg. **22**:99, 1951.

229. ROSENBERG, B. F., SPJUT, H. J., and GEDNEY, M. M.: Exfoliative cytology in metastatic cancer of the lung. The New England J. of Medicine **264**:226, 1959.

230. SALZER, G., WENZEL, M., JENNY, R. H. and STANG, L. A.: Das Bronchuscarcinom. Wien, 1952, Springer.

231. SANDERUD, K.: Squamous metaplasia of the respiratory tract epithelium -an autopsy study of 214 cases. I. incidence, age and sex distribution. Acta Path. Microbiol. Scand. **42/3**: 247, 1958.

232. SANO, M. D.: The diagnostic value of tissue culture studies of pleural effusions. Surg. etc. **97**:665, 1953.

233. SAPHIR, O.: Cytologic diagnosis of cancer from pleural and peritoneal fluids. Am. J. Clin. Path. **19**:309, 1949.

234. SAPPINGTON, S. W. and FAVORITE, G. O.: Lung puncture in lobar pheumonia. Am. J. Med. Assoc. **191**:225. 1936.

235. SCHANDLER, F. G. and MORLOCK, H.: Thoracoscopy in diagnosis. Brit. J. Med. 983, 1938.

236. SCHIEPATTI, E.: Mediastinal lymph node puncture through the tracheal carina. Surg. Gynec. Obst. **107**: 243, 1958.

237. SCHIEPATTI, E.: La punction ganglionar mediastinica a travers del espolon traqueal. Rev. d la Assoc. Med. Argeintina **72**:95, 1958.

238. SCHLEIDEN, M. J.: The Plant. London, 1853, Hyppolyte Baillière.

239. SCHLEIDEN, M. J.: Grundzuege der wissenschaftlichen Botanik nebst einer methodolog. Einleitung. Leipzig, 1842, W. Engelmann.

240a. SCHWANN, T.: The Microscopical Researches into the Accordance in the Structure and Growth of Animals and Plants. London, 1847, Sydenham Soc.

240b. SCHWANN, T.: Mikroskopische Untersuchungen ueber die Uebereinstimmung in der Struktur und dem Wachstum der Tiere und Pflanzen. Berlin, 1839, G. D. Reiner.

241. SCHWARZ, E.: "Die Lehre von der allgemeinen und oertlichen Eosinophilie." Ergebnisse der allgemeinen Pathologie (Labarsch-Ostertag). Muenchen, 1914, J. F. Bergmann.

242. SHARP, G. S.: The diagnosis of primary carcinoma of the lung by aspiration. Am. J. of Cancer **15**:853, 1931.

243. SHILLER-VOLKOVA, N. N.: Cytological examination of sputum as a method of early detection of bronchopulmonary cancer. Vopr. onkol. (Leningrad) **4(4)**: 441, 1958.

244. SIERING, H. and ADERHOLD, K.: Leitfaden der Nativcytomorphologic maligner Tumoren. Jena, 1956, G. Fischer.

245. SINAPIUS, D. and VOLLHABER, H.: Zur Kenntnis der Pleuradeckzellen. Beitr. Klin. Tbk. **106**:165, 1951.

246a. SIRTORI, C., and TALAMAFFI, F.: Cytology in lung cancer and in heavy smokers. Acta Unio Internat. Contra Cancrum **15**.477, 1959.

246b. SIRTORI C.: Results of sputum cytodiagnosis of 150 lerified lung cancers. Atti della Soc. Lombarda di Scienze Med. e Biol. 12, n. 2, 1959.

247 SÖDERSTROM, N.: Identification of normal tissues and tumors by cytologic aspiration biopsy. Acta Societas Medicorum Upsaliensis **63**:53, 1958.

248. SMETANA, H. F.: The needle biopsy in diagnosis. Am. J. Clin. Pathol. **24**:395, 1954.

249. SOMMERBRODT, J.: Ueber Genese und Bedeutung der sog. Herfehlerzellen. Berlin. Klin. Wschr. 1025, 1889.

250. SONG, Y. S.: Symposium on spindle cells. Acta Cytologica **2**:288, 1958.

251. SPJUT, H. J., FIER, D. J. and ACKERMAN, L. V.: Exfoliative cytology and pulmonary cancer. J. Thor. Surg. **30**:6, 1955.

252. SPRIGGS, A. T. and LAJTHA, L. G.: In vitro uptake of $C^{14}$-labelled glycine by the cells of pleural and peritoneal fluid. Brit. J. of Cancer **13**:132, 1959.

253. SPRIGGS, A. T.: Malignant cells in serous effusions complicating bronchial carcinoma. Thorax **9**:27, 1954.

254. STEWART, D. and EDIN, M.: Lung puncture in acute lobar pneuomonia. Lancet 520, 1930.

255. STEWART, F. W.: The diagnosis of tumors by aspiration. Am. J. Path. **9**:801, 1933.

256. STOREY, C. F., KNUDTSON, K. P. and LAWRENCE, B. J.: Bronchiolar (alveolar cell) carcinoma of the lung. J. Thor. Surg. **26**:4, 1953.

257. STREICHER, H. J.: Cytologische Betrachtungen nach intrapleuraler Losttherapie bei 5 Faellen von carcinomatoeser Pleuritis. Langenbecks Arch. u. Dtsch. Z. Chir. **266**:50, 1950.

258. Strupler, W.: Cytologische Krebsdiagnostik aus dem Bronchialsekret. Practica oto-rhino-laryngologica **12**: 17, 1950.

259. Strupler, W.: Die Zytologie des Tracheo-Bronchialsekretes. Fortschr. H.N.O.-Heilkunde Sep. Vol. 3, 1956.

260. Terrel, E., Robertson, O. and Coggeshall, L.: Experimental pneumococcus lobar pneumonia in the dog. J. Clin. Invest. **12**:393, 1933.

261. Toaff, R. O, and Bromberg, J. M.: Cytologic changes of oral epithelium in nutritional deficiencies. Am. J. Clin. Path. **21**:536, 1951.

262. Umiker, W.: Cytology in bronchogenic carcinoma. Am. J. Clin. Path. **22**:558, 1952.

263. Umiker, W.: False negative reports in the cytologic diagnosis of cancer of the lung. Am. J. Clin. Path. **28**: 37, 1957.

264. Umiker, W.: The use of chymotrypsin for the concentration of sputum in the cytologic diagnosis of lung cancer. Univ. of Michigan Med. Bull. **24**:265, 1958.

265. Valentine, E. H.: Squamous metaplasia of the bronchus. Cancer **10**:2, 1957.

266. Virchow, H.: Ernaehrungseinheiten und Krankheitsherde. Arch. f. Path. Anat. Phys. u. Kl. Med. **4**:375, 1852.

267. Virchow, H.: Die Cellularpathologie in ihrer Begruendung auf Physiologische und Patholog. Geschwuelste. Berlin, 1858, Aug. Hirschfeld.

268. Walshe, W. H.: On the Nature and Treatment of Cancer. London, 1846, Taylor and Walton.

269. Wandall, H. H.: A study on neoplastic cells in sputum. Acta chir. Scand. (Stockh.) 91, Suppl. 93, 1. 1944.

270. Watson, W. L., Cromwell, L. and Papanicolaou, G. N.: Cytology of bronchial secretions. J. Thor. Surg. **18**:113, 1949.

271. Weiss, A., Blum, E., Ondet, P. and Asch. C.: Le diagnostic histologique des cancers bronchique par biopsies et aspirations endobronchiques. Strasbourg med. **13**:410, 1952.

272. Wethered, F. J.: Medical Microscopy. London, 1893 Blakiston and Son.

273. Widal, F. and Ravaut, P.: De l'étude histologique des épanchements séro-fibrineux de la plèure. (pleurésies tuberculeuses) Bull. Soc. Biol. Paris, Ser. II. **52**:649, 1900.

274. Widal, F. and Ravaut, P.: Pleurésie dite idiopathique tuberculeuses. Compt. rend. Soc. Biol. Paris. Ser II. **52**:653, 1900.

275. Wihman, G.: Persoenliche Mitteilung. Zur Frage der Riesenzellen in Exsudaten.

276. Wihman, G.: A contribution to the knowledge of the cellular content in exudates and transudates. Acta med. Scand. (Stockh.) 130, suppl. 205, 1948.

277. Wihman, G. and Bergstrom, J.: Histological technique for the examination of the cell content of sputum. Acta med. Scand. (Stockh.) **142**:433, 1952.

278. Williams, W. J.: Alveolar metaplasia; its relationship to pulmonary fibrosis in industry and the development of lung cancer. Brit. J. of Cancer **11**:30, 1957.

279. Wittekind, D. and Struder, R.: Beitrag zur Histogenese des Bronchialkarzinoms. Frankf. Z. Path. **64**: 294, 1953.

280. Wittekind, D. and Volker, A.: Untersuchung an Erguessen seroeser Hoehlen mit dem kombinierten Phasenkontrast-Fluoreszenz-Mikroskop. Zeitschr. f. Klin. Med. **155**:403, 1958.

281. Wolf, J. and Winkhaus, R.: Das Pneumothoraxexudat unter besonderer Berueckschichtigung der Zytodiagnosnostik. Dtsch. med. Wschr. 826, 1951.

282. Wood, D. A.: Applicability of pulmonary cytology. Is surgery indicated on the basis of cellular evidence? Acta Unio Internat. Contra Cancrum **14**:509, 1958.

283. Woolf, C. R.: Application of aspiration lung biopsy with a review of the literature. Diseases of the Chest **25**:286, 1954.

284. Woolner, L. B. and McDonald, J. R.: Diagnosis of carcinoma of the lung. J. Am. Med. Assoc. **139**:497, 1949.

285. Woolner, L. B. and McDonald, J. R.: Bronchogenic carcinoma: diagnosis by microscopic examination of sputum and bronchial secretions; preliminary report. Proc. Staff. Meet. Mayo Clin. **22**:369, 1947.

286. Woolner, L. B. and McDonald, J. R.: Cytologic diagnosis of bronchogenic carcinoma. Am. J. Clin. Path. **19**:765, 1949.

287. Woolner, L. B. and McDonald, J. R.: Cytology of sputum and bronchial secretions: studies on 588 patients with miscellaneous pulmonary lesions. Ann. Int. Med. **33**:1164, 1950.

288. Woolner, L. B. and McDonald, J. R.: Cytologic diagnosis of bronchogenic carcinoma. Dis. of the Chest **17**:1, 1950.

289. Woolner, L. B. and McDonald, J. R.: Carcinoma cells in sputum and bronchial secretions. Surg. etc. **88**:273, 1949.

290. Wuhrmann, F.: Zur Diagnostik von Geschwuelsten aus Punktaten und Sekreten. Muenchener med. Wschr. **84**:860, 1937.

291. Zadek, T.: "Methodik u. Technik der Zytodiagnostik." Handbh. d. Allgem. Haematologie. Vol 2/1, Berlin, 1933, Urban and Schwarzenberg.

292. Zadek, T.: "Die Cytologie der Exsudate und Transsudate." Handbuch der allgemeinen Haematologie. Bd. I. 2. Haelfte. 1933.

293. Zatuchni, J., Campbell, W. W. and Zanafonatis, J. D.: Pulmonary fibrosis and terminal bronchiolar (alveolar cell) carcinoma in sclerodermia. Cancer (N.Y.) **6**:1147, 1953.

294. Zemansky, A. P.: The examination of fluids for tumor cells. Am. J. Med. Sci. **175**:489, 1928.

ACTA CYTOLOGICA • 1960

Vol. IV ORIGINAL ARTICLE Nos. 2 & 3

# Comparative Studies on the Degree of Proliferation of the Vaginal and Ectocervical Epithelium in the Hormonal Evaluation of a Patient by Means of Exfoliative Cytology

HANS-JÜRGEN SOOST*

## INTRODUCTION

The hormonal evaluation by means of vaginal cytology is, in principle, based upon the assessment of the degree of proliferation of the epithelium; it is evaluated from the type of exfoliated cells (parabasal, intermediate or superficial cells). In addition, there are finer criteria, applied especially to the highly proliferated epithelia, such as the turgescence of the exfoliated cells and their position in regard to each other. The cells may be flat or folded; they may be found lying singly or in clusters. And finally, factors other than hormonal have to be taken into account (i.e., bacterial flora, inflammatory changes, Trichomonas infestations, bacterial cytolysis or degenerative autolysis) which may either disturb the pattern or, on the other hand, may give certain tentative indications as to the probable hormonal situation.

Most important is the proper assessment of the degree of proliferation. This is very easily done in those cases where all the cells seem to originate from one and the same epithelial layer and in which the cellular pattern is identical in all portions of the vagina. This, however, is not always the case. On the contrary, when taking cytological specimens from various sites of the vagina or the ectocervix, one may observe considerable difference in cellular patterns, in regard to the type of exfoliated epithelial cells as well as in regard to other characteristics of the cytological specimen. Therefore, if one were not to consider the particular circumstances under which the specimens were prepared, one could arrive at divergent hormonal evaluations on various specimens taken from the same patient at the same time.

It is the purpose of this paper to investigate the differences in the degree of proliferation according to the site from which the cytological specimens were prepared.

## MATERIAL AND METHOD

Cytological specimens were prepared from 250 women with various hormonal conditions from the following sites: (1) from the ectocervix, (2) from the posterior fornix of the vagina, (3) from the proximal third of the lateral vaginal wall and (4) from the distal third of the vagina. In order to avoid even minute lacerations of the epithelium and to prevent contamination with vulvar cells, the cytological smears were prepared before the bimanual gynecological examination. After visualization of the cervix, with the aid of two specula, the specimens were taken either with an ordinary wooden spatula or with a dry or wet cotton swab. The same method of preparation of the specimens was used in each patient to permit proper comparison of the obtained results. The cytological specimens were fixed in ether-alcohol and subsequently stained according to the Papanicolaou method.

## MODUS OF REPORTING FINDINGS

For reporting the findings the nomenclature was used which has been suggested as the result of an International Symposium of Terminology (*Acta Cytologica* Vol. 2, No. 1, 25–139, 1958). Accordingly, "superficial cells" refer only to those large polygonal cells which display pyknotic nuclei. Their cytoplasm may be either eosinophilic or cyanophilic. The large polygonal cells with vesicular nuclei, which previously had been attributed to the lower layer of the superficial cells by many authors, are now classified as "intermediate cells." Thereby the group of "intermediate cells" embraces a great number of cells of different sizes and shapes. Since not all of them correspond to the same degree of epithelial maturation, or the same hormonal condition, we supplemented the above terminology and differentiate between "large" and "small" intermediate cells. The "large intermediate cells" completely resemble the "superficial cells," the only difference being that they have vesicular nuclei. The "small intermediate cells" merely exhibit a smaller size and correspond to the cells which have up to now been considered "intermediate cells" by many authors.

The assessment of the degree of proliferation has been done according to the classification of Schmitt (7), which has been supplemented by the classification of Wied (11) for identification of folding and crowding of cells. In order to make the findings more objective, indices of the various cellular forms, as well as the Karyopyknotic and the Eosinophilic Indices, were counted in some of our cases. To explain the classification of Schmitt, the diagram (Figure 1) is included with the modified cell designation according to the new nomenclature. The classification of Wied is explained in Figure 2.

* From the Department of Obstetrics and Gynecology of the University of Munich, Germany (Chairman: Professor W. Bickenbach).

Acknowledgment: The author wishes to thank his cytotechnologist, Miss Ruth Gibbert, for her assistance in the interpretation of the cytologic specimens.

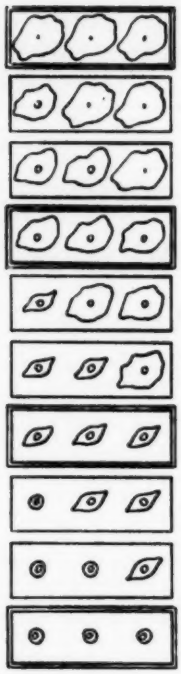



FIG. 1. Diagram to illustrate the classification into degrees of proliferation in vaginal smears according to Schmitt (7), with modified cell designations according to the newly adopted nomenclature (*Acta Cytologica* 2:1, 25–139, 1958).

| | | |
|---|---|---|
| A | 100% flat cells | |
| B | 75% flat cells, | 25% folded cells |
| C | 50% flat cells, | 50% folded cells |
| D | 25% flat cells, | 75% folded cells |
| E | | 100% folded cells |

| | | |
|---|---|---|
| a | 100% single cells | |
| b | 75% single cells, | 25% crowded cells |
| c | 50% single cells, | 50% crowded cells |
| d | 25% single cells, | 75% crowded cells |
| e | | 100% crowded cells |

FIG. 2. Diagram for the assessment of folding and crowding of cells, according to Wied (11). "A" means that 100% of all cells are flat. At "C" approximately 50% of all cells are flat, whereas the other 50% are folded. "E" means that all cells are folded, with the intermediate degrees "B" and "D" as shown in the figure. The percentage of flat or folded cells is estimated. The assessment of single and crowded cells is done analogously in "a" to "e".

## RESULTS

The results of the investigation shall be discussed within the following four groups:

I. Specimens exhibiting uniform proliferation of the epithelium at all sites in the vagina and on the ectocervix;

II. Specimens exhibiting increased proliferation in the distal third of the vagina;

III. Specimens exhibiting increased proliferation on the ectocervix;

IV. Specimens exhibiting mixed patterns with cells of all layers at all sites of the preparation.

The frequency in which the various forms of proliferation were encountered is shown in Table I.

TABLE I

| Degree of proliferation (according to Schmitt) | 1 | 1–2 | 2–1 | 2 | 2–3 | 3–2 | 3 | 3–4 | 4–3 | No. of Cases |
|---|---|---|---|---|---|---|---|---|---|---|
| No unequivocal differences between the various sites of proliferation | 1 | 2 | 1 | 3 | 8 | 11 | 32 | 78 | 11 | 147 |
| Higher proliferation in the distal third of the vagina than on the lateral vaginal wall or on the ectocervix | 7 | 14 | 6 | 10 | 4 | 2 | 4 | ..... | ..... | 47 |
| Higher degree of proliferation on the ectocervix than on the lateral vaginal wall | ..... | ..... | ..... | 2 | 2 | 12 | 12 | 7 | 2 | 37 |
| Lower degree of proliferation in the distal third of the vagina than on the lateral vaginal wall. (+ means at the same time higher degree of proliferation at the ectocervix; already listed in the previous group) | ..... | ..... | ..... | ..... | ..... | ..... | (+1) 2 | (+3) 4 | 4 | 10 |
| Mixed cellular patterns from all sites of smear preparation | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 9 |
| Total | 8 | 16 | 7 | 15 | 14 | 25 | 50 | 89 | 17 | 250 |

TABLE I. Results of comparative studies on smears of 250 women taken from various sites. The results show that in cases of highly proliferated vaginal epithelium (Degrees 3 to 4 and 4 to 3 of Schmitt), differences in the smear pattern from the various sites are relatively rare. In cases of atrophic or only slightly proliferated vaginal epithelium (Degree 1 to 2 of Schmitt) one finds almost regularly a higher proliferated epithelium in the distal third of the vagina. In cases of intermediate proliferation (Degrees 2 to 3 or 3 of Schmitt) the findings change: the proliferation is partly the same at all sites, and partly elevated on the ectocervix or on the distal third of the vagina.

## I. SPECIMENS EXHIBITING UNIFORM PROLIFERATION OF THE EPITHELIUM AT ALL SITES OF SMEAR PREPARATION IN THE VAGINA AND ON THE ECTOCERVIX

Practically identical cellular patterns at the various sites have been found primarily in women during the reproductive age, during both the follicular and the luteal phases. In the majority of cases the Proliferation Degrees were "3 to 4" or "4 to 3" (of Schmitt). Among 106 women with proliferation of the vaginal epithelium up to this height, differences were found in only 17 according to the various sites of smear preparation. The tendency was towards a higher proliferation on the ectocervix and a lower proliferation on the distal third of the vagina.

In women with intermediate proliferation of the epithelium (Degrees "2 to 3" or "3" of Schmitt) and with an atrophic epithelium (Degrees "1" or "2" of Schmitt) we found uniform cellular patterns at the various sites with less regularity. In women with Proliferation Degrees "2 to 3" or "3", the proliferation corresponded at the various sites in only 51 of the 89 cases. In 38 cases there was a difference: the proliferation was higher in the distal third of the vagina in some cases and in the ectocervix in some cases.

Among 46 women with Proliferation Degree "1 to 2" (of Schmitt) only seven showed uniform proliferation. In 39 the proliferation was different at the various sites of preparation. With the exception of two cases, a higher proliferation was noted in the distal third of the vagina.

One sometimes finds, in cases of uniform epithelial proliferation, minute differences among the individual smears, in most cases concerning merely the degrees of cellular folding and crowding. One will not be able in every case to attribute any significance to these findings. Some deviations belong, without doubt, to the physiological range of cytological patterns. Oftentimes they may be explained by artificial influences during the preparation of the smears, which cannot always be avoided even with the most careful technique. Very minute differences of such kinds have been disregarded in our investigations.

## II. SPECIMENS EXHIBITING PROLIFERATION IN THE DISTAL THIRD OF THE VAGINA

In post-menopausal women with an atrophic cell pattern (Proliferation Degrees "1" or "1 to 2") or with only moderately proliferated vaginal epithelium (up to Proliferation Degree "2 to 3") we found, almost regularly, higher epithelial proliferation in the distal portions of the vagina.

Case No. 1 (Fig. 3, *abc*): 52 year old patient; three years post-menopausal after radium-menolysis, showed the pattern of Proliferation Degree "1" on the ectocervix, Degree "1 to 2" on the lateral vaginal wall, and Degree "3 to 2" in the distal third of the vagina.

The computation of the cellular indices is shown in Figure 4. The Karyopyknotic Index corresponds to the Superficial Cell Index. The percentage of eosinophilic cells among the superficial cells (Eosinophilic Index) is marked in the column of the superficial cells by a transverse bar.

Among 31 cases with the Proliferation Degrees of "1" and "1 to 2" on the lateral vaginal wall, we observed, in 27 cases, a markedly higher proliferation in the distal third of the vagina, at times up to Proliferation Degree "3 to 4". In four cases no differences were observed. In these latter cases two patients were post partum.

Among the 74 cases with the Proliferation Degrees of "2" up to "3" on the lateral vaginal wall, higher proliferation in the distal third of the vagina was found in only 20 cases, while proliferation was the same at both sites in the other 54 patients.

From the Proliferation Degree "3 to 4" on up, no cases with a higher degree of proliferation towards the vaginal introitus could be found, and in some cases the proliferation there was lower. A detailed report of the findings is given in Table II.

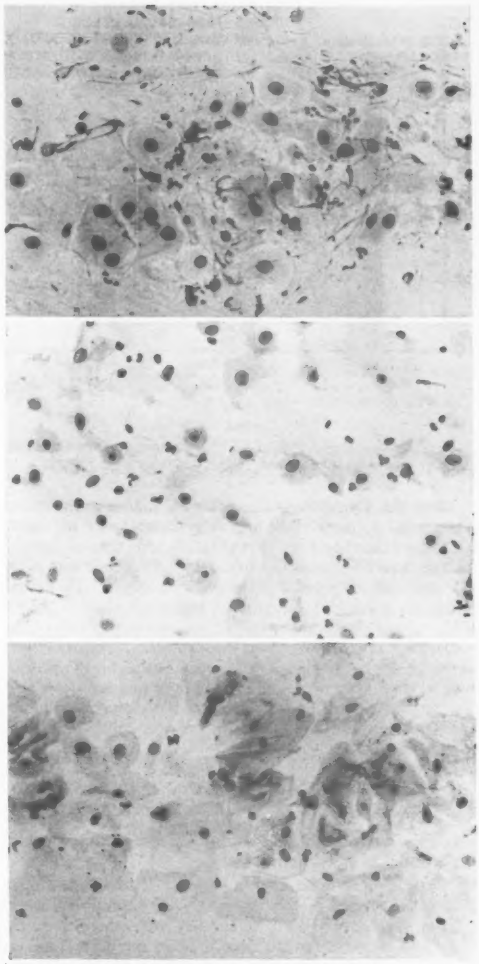


FIG. 3—*abc*. Cytological specimens prepared from Case No. 1, and taken from different sites; ectocervical smear (*top*): almost exclusively parabasal cells; lateral vaginal wall (*center*): predominantly parabasal cells, and in addition there are "small" and single "large" intermediate cells; distal third of the vagina (*bottom*): predominantly "large" intermediate cells, fewer "small" intermediate cells and single parabasal and superficial cells.

How can these differences in the proliferation be explained? One could assume that the distal portion of the vagina has a higher sensitivity to hormonal stimuli. Consequently, in that case, the epithelium in the distal third of the vagina should show an especially marked proliferation after administration of estrogens. However, we could not observe this in any case. On the contrary, the vaginal epithelium in the distal portion of the vagina reacted even less markedly to hormonal stimuli than the proximal vaginal portion or even the ectocervix. Even when the pattern was initially characterized by a relatively high degree of proliferation of the epithelium around the vaginal introitus, this was soon surpassed by the more marked proliferation of the proximal portions of the vagina.

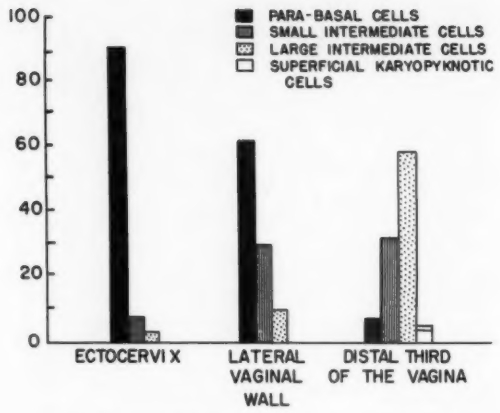



FIG. 4. Cytological indices of the specimens from Case No. 1, taken from the various sites (compare with Fig. 3, *abc*).

Thus, the almost regularly observed higher proliferation in the distal portions of the vagina in patients with hormonal deficiency cannot be due to hormonal factors. One may rather explain these findings with exogenous influences, most probably mechanical causes. Presumably, the vaginal epithelium adopts the protective function of the outer skin. Thereby, its high sensitivity to hormonal stimuli apparently decreases.

Occasionally, in such cases, the ectocervical smears exhibit markedly lower proliferation as compared to the smears from the lateral vaginal wall. Whether or not one deals with changes due to marked atrophy or with a disturbance of the cytological pattern by so-called "erosion cells" could not be decided.

## III. SPECIMENS EXHIBITING INCREASED PROLIFERATION OF THE ECTOCERVIX

Higher degrees of proliferation on the ectocervix were found under two conditions:

A. When one administers low dosages (just above the threshold dosage) of a proliferation-inducing hormone to women with an initially atrophic cellular pattern, one frequently sees a very marked proliferation on the ectocervix and, temporarily, a more marked hormonal effect than the lateral vaginal wall. On the other hand, in the distal third of the vagina the epithelium often shows less response.

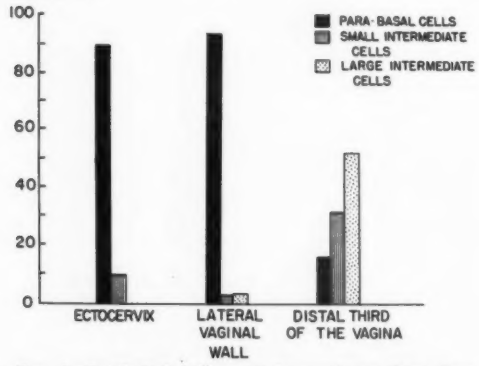



FIG. 5. Cytological indices of the specimens from Case No. 2, taken from the various sites before administration of estrogen (compare with Fig. 6, *abc*).

**TABLE II**

PROLIFERATION DEGREES IN THE DISTAL THIRD OF THE VAGINA

| PROLIFERATION DEGREES ON THE LATERAL VAGINAL WALL | 1 | 1-2 | 2-1 | 2 | 2-3 | 3-2 | 3 | 3-4 | 4-3 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | | 1 | 3 | 2 | 1 | | | |
| 1-2 | | 2 | 1 | 3 | 3 | 4 | 2 | 1 | |
| 2-1 | | | 1 | | 3 | 2 | 1 | | |
| 2 | | | | 3 | 3 | 4 | 2 | 1 | |
| 2-3 | | | | | 8 | | 2 | 2 | |
| 3-2 | | | | | | 11 | 1 | 1 | |
| 3 | | | | | 1 | 2 | 32 | 4 | |
| 3-4 | | | | | 1 | 2 | 4 | 78 | |
| 4-3 | | | | | | | 1 | 3 | 11 |

TABLE II. Compilation of 47 cases with higher proliferation in the distal third of the vagina grouped according to the proliferation Degrees of Schmitt (to the right of the framed diagonal column of figures). For comparison, the number of cases with identical degree of proliferation at all sites of preparation (heavily framed diagonal column of figures), or with decreased proliferation in the distal third of the vagina (to the left of the heavily framed diagonal column of figures) are shown. One recognizes at the same time which degrees of differences of proliferation occur at the various sites.

Case No. 2: 56 year old patient; seven years postmenopausal, showed on three cytological examinations at intervals of three days each, atrophic cell patterns. The smear taken from the ectocervix showed Proliferation Degree "1", the one taken from the lateral vaginal wall Proliferation Degree "1", and the smear from the distal third of the vagina, Proliferation Degree "2-3". The indices computed from the individual smears are demonstrated in Figure 5.

The patient received 2.5 mg. diethyldioxystilben dipropionate intramuscularly. On the seventh day the following cytological patterns were found: ectocervix: Degree "3 to 4"; lateral vaginal fornix: Degree "3"; distal third of the vagina: Degree "3 to 2" (Fig. 6, *abc*).

While the proliferation prior to hormone administration was clearly the highest in the distal third of the vagina, it was found to be the lowest at this site after the administration. The comparison of the specimens prepared from the various sites pointed to a continuous decrease of epithelial sensitivity to hormonal stimuli from the ectocervix towards the vaginal introitus. This fact is expressed in the cytological indices computed in Figure 7.

B. Without administration of hormones one finds similar differences at the various sites, especially in smears displaying moderate degrees of proliferation, such as in women with existing, but not fully pronounced, follicular hormonal activity. While smears from the lateral vaginal fornix show relatively small, folded and crowded cells, the ectocervical smears contain large, flatter and finer cells. The exact determination of the Karyopyknotic and Eosinophilic Indices disclosed that we did not deal merely with varying degrees of folding phenomena, but that true differences in the degree of maturation of the cell patterns were present.

Case No. 3: a 46 year old patient who has irregular, sometimes prolonged, vaginal bleedings at intervals of six to 12 weeks. The specimen from the lateral vaginal wall showed the Proliferation Degree "3Cb" (see Figure 8a), from which one may conclude slight estrogen deficiency. From the ectocervical smear, which we classified "3 to 4 Ba" because of its predominantly flat-lying,

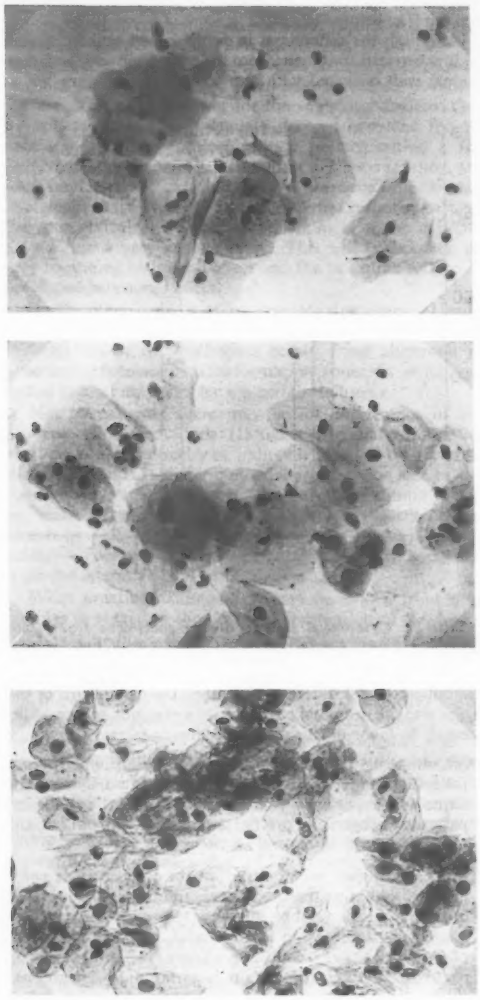


FIG. 6—*abc*. Case No. 2: Photomicrograph of cytological specimen taken from various sites on the seventh day after administration of 2.5 mg. diethyldioxystilben dipropionate. Ectocervical smear (*top*): large eosinophilic and cyanophilic intermediate cells, numerous superficial cells with pyknotic nuclei. Smear from the lateral vaginal wall (*center*): predominantly "large" intermediate cells, increased cyanophilia. Specimen from distal third of the vagina (*bottom*): predominantly "large" and "small" intermediate cells.

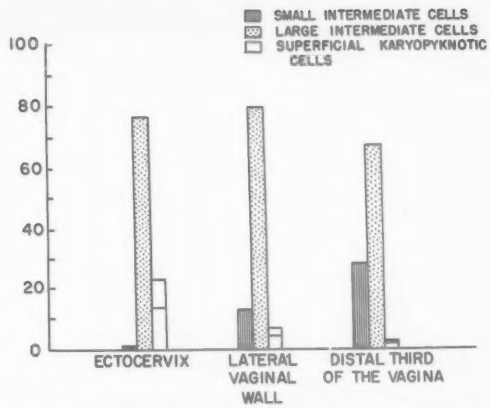



FIG. 7. Cytological indices of the specimens from Case No. 2, taken from the various sites after estrogen administration.

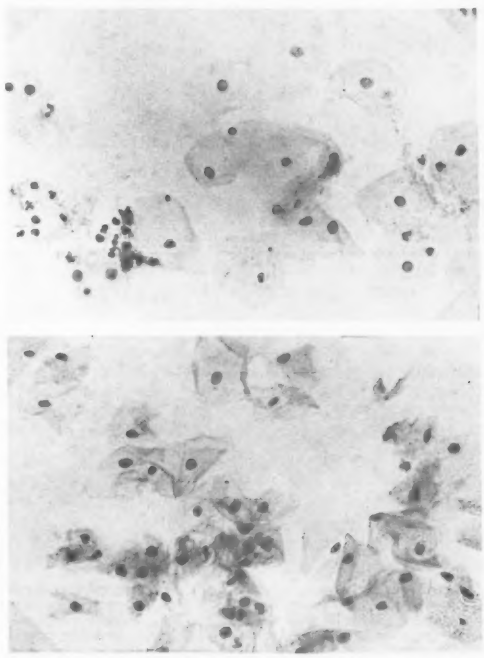


FIG. 8—*a, b*. Photomicrographs of the cytological specimen of case No. 3 taken from various sites. Ectocervical smear (*top*): large flat-lying, intermediate cells and single, superficial cells, predominantly eosinophilic and lying singly. Specimen from the lateral vaginal wall (*bottom*): "large" and "small" cyanophilic and eosinophilic intermediate cells, almost without exception folded and partly crowded.

eosinophilic cells, one could conclude good estrogenic effect. The individual cytological indices can be seen in Figure 9. An endometrial curretage performed the same day revealed an irregularly and insufficiently proliferated endometrium without any signs of secretory activity.

Case No. 4: a 34 year old patient, 18th day of menstrual cycle, elevation in basal body temperature two days previously. One observes Proliferation Degree "3 — 4 Ec" in the lateral vaginal wall, and Proliferation Degree "3 — 4 Bb" on the ectocervix (Fig. 10, *abc*). The cytological indices are presented in Figure 11. While one may assume that the smear from the lateral vaginal wall exhibits good progestational effect because of the marked crowding and folding of the cells, the smear from the ectocervix gives the impression of striking estrogenic effect. The endometrial currettage yielded early secretory changes.

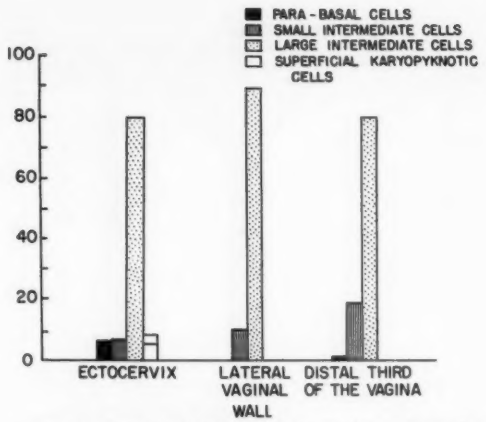



FIG. 9. Cytological indices of the specimens from Case No. 3, taken from the various sites (compare with Fig. 8, *ab*,).

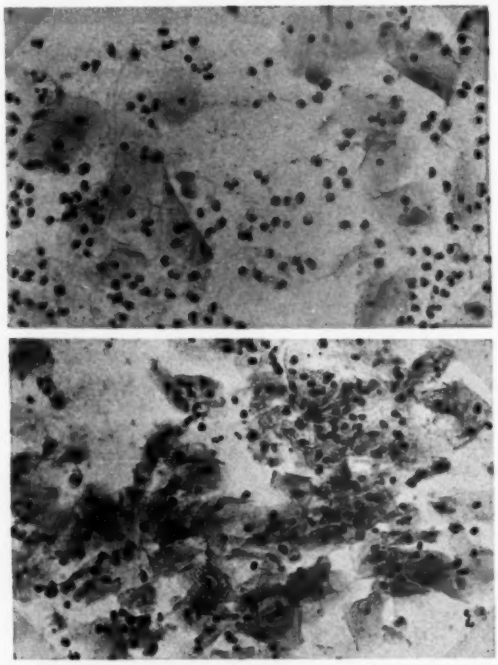


FIG. 10—*a, b.* Photomicrographs of the cytological specimens of Case No. 4 taken from the various sites. Ectocervical smear (*top*): superficial cells and "large" intermediate cells, predominantly eosinophilic, flat and lying singly. Smear from the lateral vaginal wall (*bottom*): "large" intermediate and superficial cells, single, "small" intermediate cells, however, exclusively folded and lying crowded in dense clusters.

Among 25 cases with the Proliferation Degree "3 to 2" on the lateral vaginal wall (see Table I), we observed, in 12 cases, a higher degree of proliferation at the ectocervix. Among 50 cases with the proliferation Degree "3" this observation was made in 12 cases. Much less frequently we found differences between the ectocervical and vaginal smear in cases of a fully mature epithelium, namely only in nine cases out of 106 cases with the Proliferation Degrees "3 to 4" and "4 to 3". We never observed a higher proliferation on the ectocervix in cases of an atrophic vaginal epithelium (i.e. below Degree "2").

Although the differences in proliferation and in cellular folding between specimens from the ectocervix and the lateral vaginal wall occurred with less regularity and were not as

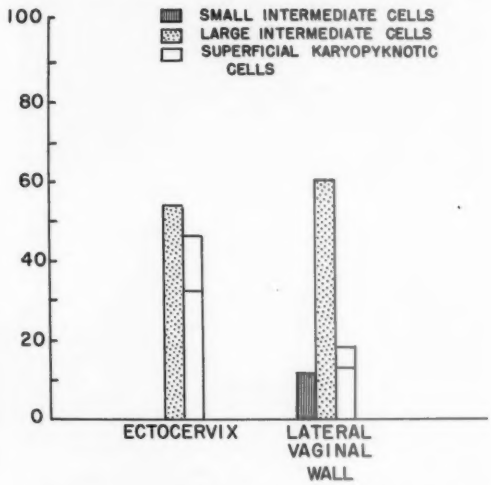



FIG. 11. Cytological indices of the specimens from Case No. 4, taken from the various sites (compare with Fig. 10, *a,b*).

pronounced as the differences between the distal third of the vagina and the proximal portions, they may not be purely incidental. In favor of this are the following facts:

1. Simultaneous preparation of cytological specimens from the left and right vaginal fornix demonstrated, if the technique of preparation was correct, identical cytological patterns in regard to proliferation and positioning of the exfoliated cells, in almost all the cases.
2. Markedly higher proliferation on the ectocervix as compared with that on the lateral vaginal fornix is encountered in only a small number of cases. However, the opposite has never been seen in our material, with the exception of some cases of atrophic or very lowly proliferated cellular patterns. We are not certain how far a low hormonal activity is simulated by the presence of "erosion cells" in these cases.
3. The same is true for the occurrence of cellular folding and crowding which is almost always found to be increased in the specimens prepared from the lateral vaginal wall in those cases where differences between the ectocervix and the lateral vaginal wall were present.

The difference in proliferation is even more pronounced when one prepares the specimen (in those patients with a high endocervical squamo-columnar junction), not from the ectocervix, but from the distal portion of the endocervix. Smears from the posterior vaginal fornix displayed, in most cases, less pronounced differences when compared with ectocervical smears. This may be due to the fact that in the posterior fornix, cells from all other parts of the vagina and the genital tract are collected. On the other hand, one may not always be dealing with freshly exfoliated cells, as compared with the specimens from the lateral vaginal wall.

The increased proliferation of the ectocervix is difficult to interpret. Since it appears after administration of estrogens (as shown in Case No. 2) one is tempted to attribute this to a marked hormonal sensitivity of the ectocervical

epithelium. Differences in proliferation between the vaginal and ectocervical epithelium do not occur regularly. This may be due to certain peculiarities, depending upon how the vaginal epithelium reacts. The atrophic cellular pattern disappears as soon as a certain hormonal threshold dosage is reached. This value is lower for the vaginal epithelium than for the endometrium. Adequate dosages provided for the development of the typical cellular pattern within a few days. Once this stage of proliferation has been reached, any additional amount of administered hormone will not add to changes in the pattern. Thus, a different response between the ectocervical and vaginal epithelium is to be expected only within a very narrow range. This will be between the very beginning hormonal effect and the not quite fully developed hormonal effect.

On the other hand, it seems possible that chemical irritation by cervical secretions plays a certain role, or that a higher stratification of the epithelium results from abnormal regenerative processes in transformation zones, in which hormonal stimuli may also have a certain influence.

Not infrequently there may be an interference of two processes of different kinds: (1) higher epithelial proliferation on the ectocervix directly or indirectly released by hormonal stimulation and (2) higher epithelial proliferation in the distal third of the vagina caused by non-hormonal stimuli. Thereby, series of smears may result in which the ectocervical smears as well as the specimens from the vaginal introitus exhibit higher proliferated cell patterns than the specimens from the lateral vaginal wall.

What practical consequences can be drawn from these findings in regard to the hormonal evaluation of cytological specimens? Above all, one asks oneself whether or not the evidence of estrogenic or progrestational activities are equally well expressed in different portions of the vagina. This is not the case. One is able to diagnose a considerably higher degree of estrogenic effect from the cervical smear as compared with the specimen from the lateral vaginal wall. In cases of only slight or beginning progestational activity, the cytological criteria (i.e., folding and crowding) for this condition are often absent in the ectocervical smear, whereas these criteria can be recognized very well in specimens from the lateral vaginal wall. There can be no doubt that the cytological changes due to the progrestational activity appear most clearly in specimens from the lateral vaginal wall. Even in cases with marked follicular hormone stimulation there is a tendency towards folding and crowding of cells in the specimens from the lateral vaginal wall, as contrasted with those prepared from the ectocervix. In other words, the ectocervical epithelium seems to possess a pronouncd teendency towards exfoliation of large, flat, single-lying cells, apparently independent of the hormonal condition. This has to be kept in mind in order to avoid being misled by deviations of the cellular patterns which are due to the preparation technique, especially in serial studies. The best site for taking smears for hormonal evaluation is, without doubt, the lateral vaginal wall, because here one finds the least disturbance by non-hormonal factors.

## IV. SPECIMENS EXHIBITING MIXED PATTERNS WITH CELLS OF ALL LAYERS AT ALL SITES OF PREPARATION

As the last group, the cytological patterns of the "mixed type" are mentioned, containing cells from all layers of the epithelium in various, relative amounts, from the eosinophilic superficial cells to the parabasal cells. Three causes for the occurrence of these cytological patterns are possible and shall be dealt with in the sequence of their frequency:

(1) One may deal with so-called "erosion patterns". In cases of true erosions, ectopies or insignificant lacerations of the epithelium, frequently single-lying or grouped parabasal cells are exfoliated and less frequently intermediate cells are seen. These cells have nothing to do with estrogen deficiency. Only the cells from the upper layers of the epithelium are useful for the hormonal evaluation of the smear. It is characteristic that the so-called "erosion cells" occur in large numbers mainly in the ectocervical specimens, whereas in smears from the lateral vaginal wall they are rare or even completely absent. This is also one of the main reasons why ectocervical specimens are unfit for hormonal evaluations. By no means should one obtain material for hormonal evaluations by means of the Ayre spatula: with this technique one may forcefully scrape cells from deep layers of the epithelium.

In cases of marked epithelial proliferation, single "erosion cells" can easily be recognized as such (Fig. 12). If there is an abundance of parabasal cells present or there is a low degree of proliferation in a given smear, then a "pseudo-estrogenic deficiency" may be easily simulated (Fig. 13).

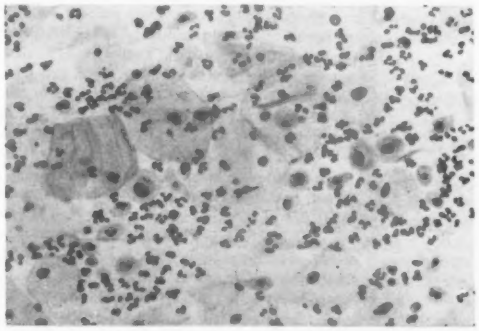


FIG. 12. Highly proliferated cytological pattern with single parabasal cells which one may recognize as "erosion cells" (Proliferation Degree "3 to 4").

(2) In cases with low estrogen effect a mixed cytological pattern may result when higher proliferated cells from the area of the introitus and the vulva are transferred into the vagina by digital gynecological examination, douches, or cohabitation. Then one will find, in addition to predominantly parabasal cells, single intermediate and superficial cells from the upper epithelial layers and also anucleated squames which in no way fit in with the rest of the pattern. To avoid errors of this kind the smears should always be taken prior to the digital gynecological examination.

(3) In addition, there are cytological patterns of the mixed type that may be caused by hormonal deficiency of various degrees. If one administers estrogens to a women exhibit-

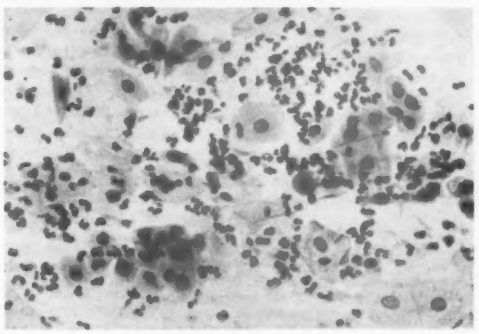


FIG. 13. "Erosion cells" in a cytological specimen with low epithelial proliferation, which may simulate an atrophic pattern (Proliferation Degree "2 to 3").

ing the atrophic cytological pattern, then the highly proliferated cellular pattern does not necessarily develop via a scale of uniformly exfoliated intermediate patterns. In many cases, between the atrophic and the markedly estrogenic stage of proliferation, one encounters cells from all epithelial layers in various relative amounts.

Case No. 5: 67 year old patient, 13 years post-menopausal, showed the atrophic cellular pattern with predominantly parabasal and "small" intermediate cells (Proliferation Degrees "1" or "2 to 1"). There was a higher degree of proliferation of the epithelium towards the introitus of the vagina. The exact indices are shown in Figure 14.

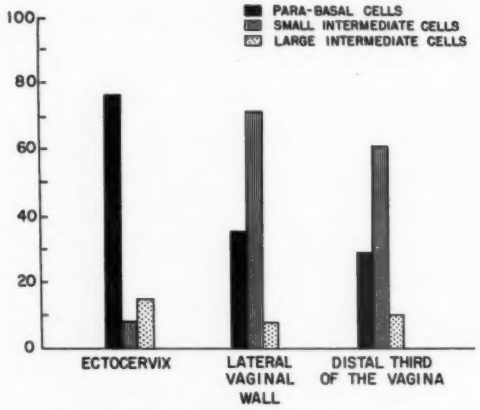



FIG. 14. Cytological indices of the specimens of Case No. 5, taken from the various sites before administration of estrogens

On the third day after intramuscular administration of 2.5 mg. estradiol butyrylacetate, we found in all specimens the mixed cellular pattern consisting of approximately the same composition, containing parabasal, "small" and "large" intermediate, as well as superficial cells (Proliferation Degrees "1" up to "4"). The cytological indices from the various sites of preparation are shown in Figure 15.

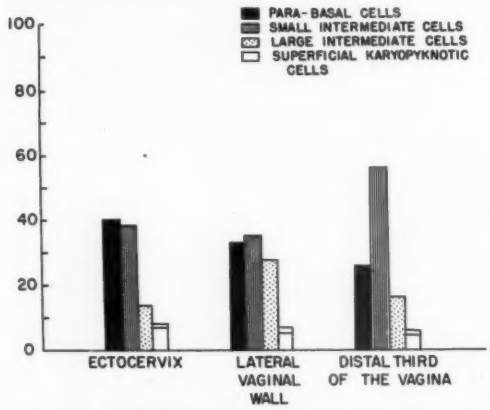



FIG. 15. Cytological indices of the specimens from Case No. 5, taken from the various sites on the third day after administration of estrogen.

On the seventh day after administration of the hormone the proliferation in the different smears was again even, in so far as there was again the predominance of one particular cellular layer. The degree of proliferation was "3 Cd" on the lateral vaginal wall, and approximately "3 to 4 Bb" on the ectocervix. The exact cytological indices are presented in Figure 16. In characteristic fashion the ectocervical specimen here displayed criteria of higher estrogenic activity than the specimen from the lateral vaginal wall.

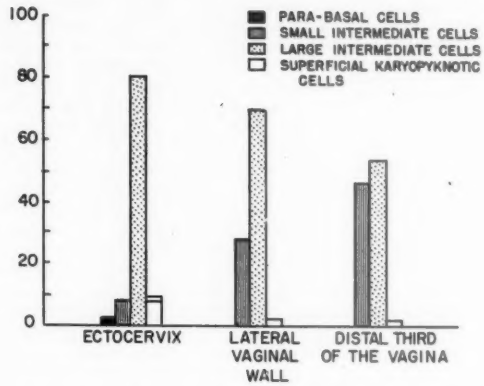



FIG. 16. Cytological indices of the specimens from Case No. 5, taken from the various sites on the seventh day after administration of estrogens.

Similar cellular patterns from the mixed type, as shown in Figure 15, are also found when the hormonal stimulus ceases. Sometimes these patterns may be seen for a longer period of time during the beginning of menopause. Women with atrophic vaginal epithelium and a high endocervical squamo-columnar junction may show, very shortly after hormonal administration, a mixed cellular pattern in the distal portions of the endocervix in which single superficial cells lie side by side with parabasal and intermediate cells; whereas in the vaginal specimens uniformly low proliferative patterns predominate. These cellular patterns of the mixed type, in cases with insufficiently developed epithelial growth, apparently result from the uneven surface of the connective tissue. The epithelium above the valleys of the connective tissue is proliferated up to the height of the superficial cells; parabasal and "small" intermediate cells are found above the papilli of the connective tissue.

## DISCUSSION OF PREVIOUS LITERATURE

Adler (1) and Stieve (10) have already pointed to differences in the histological structure of the vaginal epithelium.

In more recent cytological literature Pundel (5) drew attention to the minor cyclic changes in the distal third of the vagina, and to relatively increased Karyopyknotic and Eosinophilic Indices found primarily in patients with uterine prolapse. The simultaneous presence of parabasal and superficial cells in the specimens was thought to be due to contamination of the vaginal smear with cells from the area of the vaginal introitus. Pundel was also the one who, in addition to other authors (8), again and again emphasized the importance of an exact preparation of the cytological smear for hormonal evaluations. For the site of preparation Pundel recommended the proximal third of the lateral vaginal wall, because the vaginal epithelium seems to respond there most accurately to hormonal stimuli.

Wied (11) counted, in 100 women, the percentage of "large" intermediate and superficial cells and the Karyopyknotic and Eosinophilic Indices in smears taken from five different sites (1 cm above the introtus, 3–4 cm above the introitus, in the vaginal fornix, on the ectocervix, and in the

endocervix). Wied found the relatively lowest figures in the smears prepared from the vaginal fornix: 63% "large" intermediate and superficial cells, Karyopyknotic Index 35, and Eosinophilic Index 23. All indices increased considerably towards the introitus, and towards the ectocervix and endocervix. One cm above the vaginal introitus, the following values were found: 98% "large" intermediate and superficial cells, Karyopyknotic Index 51 and Eosiniphilic Index 34. The corresponding values of the endocervix were: 91% "large" intermediate and superficial cells, Karyopyknotic Idnex 42, and Eosinophilic Index 32. Despite the fact that these figures represent average values from determinations of indices with a considerably wide range of individual values, one may still recognize agreement with our findings. Wied did not specify as to the age and hormonal condition of his patients. Wied's average values of the indices on specimens prepared from the area near the vaginal introitus are higher in his study than those of the ectocervical specimens. This may have something to do with the particular composition of his patient material (age, hormonal condition). Also in his findings it is striking to see the small variability in the percentages of "large" intermediate and superficial cells in the specimens prepared close to the vaginal introitus, a fact that may point to a relatively slow response of the epithelium.

Arronet (2) compared smears of the anterior fornix with those of the medial and external third of the vaginal wall. There were no effective differences among the smears from the different spots. His conclusion was that smears of all parts of the vagina are useful for hormonal diagnosis. However, due to possible disturbance by erosive defects and by contamination with extravaginal material, he suggested not taking smears from posterior vaginal fornix or from the cervix.

Opposed to our findings are the results reported by Peters et al. (3). These authors prepared comparative specimens from 125 Indian women, however only post partum and during lactation, and in the periods from one month up to one year or longer, after delivery. They found a lower degree of proliferation on the ectocervix than in the posterior vaginal fornix. The authors concluded from these findings that the ectocervical epithelium has a lesser degree of sensitivity to hormonal stimuli. We were not able to obtain similar findings, not even on the 29 women from whom cytological specimens were prepared in the period between one week and four months after delivery.

## DISCUSSION OF OWN FINDINGS

The discussion of our own findings is based on the assumption that the cells observed in the respective specimens actually originated from the site from which they were prepared. One may deem this highly probable since the findings, as described, are to be encountered regularly. However, this is by no means certain, taking into account the close contact of the vaginal walls. It would also be conceivable that cells with a certain regularity are removed from their site of origin and then would be found at other sites in their best concentration.

Definite knowledge as to the true origin of the cells could only be brought about by the histological demonstration of a significantly different cellular structure at various sites of the vaginal epithelium. However, one should keep in mind the fact that it will be much harder to demonstrate differences in cells lying within a tissue than in the exfoliated cells. One recalls in this context the histological investigations by Dierks on the cyclic changes of the vaginal epithelium which for a long time have been controversial and which finally have been confirmed by the cytological studies of Papanicolaou.

However, it may be, even if the cells seen in the cytological smears would not originate from the sites of preparation, this does not necessarily diminish the value of the comparative results of the present study. The fact alone, that differences are being found with regularity in the cytological specimens prepared from various sites of the vaginal and ectocervical epithelium, allows one to draw practical conclusions and to consider them in the hormonal evaluation of the patient by means of cytologcal specimens.

## CONCLUSIONS

Supported by comparative studies on 250 women, definite differences in the cytological patterns in various vaginal and ectocervical specimens are demonstrated. The significance of these findings for the hormonal evaluation of the patient by means of cytological examination is discussed.

The site of choice for the preparation of cytological specimens for hormonal evaluation of the patient is the proximal third of the lateral vaginal wall. At this site the cytological patterns correspond most closely to the actual hormone condition. Also, disturbing non-hormonal factors are minimal at the proximal third of the lateral vaginal wall.

In the cases where the cytological specimen is prepared from sites other than the lateral vaginal wall, the cytologist should be so informed in order to enable him to consider the differences in the cellular pattern probably resulting from the change in technique.

Specimens prepared from the distal third of the vagina show a higher degree of proliferation in women with atrophic vaginal epithelium, whereas the response after administration of hormones is relatively slow at this site.

Inversely, in ectocervical specimens taken from women during their reproductive age, after hormone administration, one frequently sees a higher degree of proliferation and a lesser degree of cellular folding and crowding. Thereby, the ectocervical specimens may give the impression of a more marked estrogenic or a less marked progestrogenic effect than those prepared from the lateral vaginal wall.

Endocervical specimens show even more pronounced features than ectocervical specimens. If their use for hormonal diagnosis is not limited by abundance of mucus and glandular cells. The specimens prepared from the posterior vaginal fornix, as the collecting pool for cells originating from various sites generally exhibit transitional stages between the differences of the vsrious sites of preparation.

Cellular patterns consisting of cells from various layers may occur as the result of epithelial lacerations, or of contamination with cells from the vaginal introitus, or as a result of hormonal deficiencies of various degrees. In the case of doubt, the cause can be found by preparation of specimens from various sites.

These relatively fine points may not be of importance in each and every case. A rough orientation concerning the basic hormonal condition is possible by means of the cytological examination from almost any site of the vaginal epithelium. For a more exact hormonal evaluation, however, especially when considering various degrees of estrogenic activity, and progestational and androgenic activity, the correct preparatory technique is important and the consideration of the differences in the cellular patterns which may result from preparation of specimens from various sites in the female reproductive tract seems indispensable.


## RÉSUMÉ

Des études comparatives portant sur une série de 250 femmes ont montré une différence significative dans les aspects cytologiques de différents frottis vaginaux et exocervicaux, et la signification de ces constatations pour l'évaluation hormonale est discutée.

Le niveau de choix pour le prélèvement de frottis hormonaux est le tiers postérieur de la paroi vaginale latérale. A ce niveau, les aspects cytologiques correspondent le mieux

à l'état hormonal présent. Les facteurs gênants non hormonaux sont reduits au minimum à cet endroit.

Si le frottis est prelévé à un autre endroit que la paroi latérale du vagin, le cytologiste doit être informé afin qu'il puisse tenir compte des différences locales possibles du changement de technique.

Les frottis prélevés au tiers antérieur du vagin montrent un degré de prolifération plus élevé chez des femmes avec atrophie vaginale, tandis que la réponse à des administrations d'hormones est plus lente à cet endroit.

D'un autre côté, les frottis exocervicaux prélevés chez des femmes encore réglées montrent fréquemment un degré de prolifération plus élevé après administration hormonale et un degré moindre de plissement cellulaire et placards. Ainsi, les frottis exocervicaux peuvent donner l'impression d'une stimulation oestrogénique plus forte ou progestéronique plus faible que les frottis prélevés au niveau de la paroi vaginale latérale.

Les frottis endocervicaux montrent des différences encore plus prononcées que les frottis exocervicaux, s'ils ne sont pas completement ininterpretable à cause de leur teneur en mucus et cellules glandulaires. Les prélèvements provenant du cul de sac postérieur de vagin qui est un endroit collecteur pour des cellules d'origine différente montrent en général des images intermédiaires entre les frottis cervicaux et vaginaux.

Des images cytologiques montrant des cellules de différentes couches peuvent avoir comme origine des déchirures épithéliales ou des contaminations avec des cellules provenant de l'entrée du vagin ou encore des déficiences hormonales à des degrés variés. En cas de doute, la cause peut être trouvée en examinant des frottis provenant d'autres endroits.

Ces quelques points de détail peuvent être sans importance dans certains cas. Une orientation grossière sur les conditions hormonales de base est possible sur les frottis provenant de n'importe quel endroit de l'épithélium vaginal. Mais pour une evaluation hormonale exacte par contre, surtout si l'on veut distinguer les différents degrés d'activité oestrogénique, androgénique ou progestéronique, une technique correcte de prélèvement est importante et il est nécessaire de prendre en considération les différences possibles des images cytologiques selon l'endroit de prélèvement.

## ZUSAMMENFASSUNG

An Hand von Vergleichsuntersuchungen bei 250 Frauen wird auf Unterschiede in den Vaginal- dnd Portioabstrichen durch Entnahme von verschiedenen Stellen hingewiesen. Die Bedeutung dieser Befunde für die hormonale Diagnostik wird diskutiert.

*Der Ort der Wahl für die Entnahme des Zellmaterials zur hormonalen Untersuchung sollte das obere Drittel der seitlichen Scheidenwand (seitliches Scheidengewölbe) sein. An dieser Stelle dürfte sich am deutlichsten das Zellbild ausprägen, welches der wirklichen Hormonlage entspricht. Die störenden, nicht hormonalen Einflüsse sind hier am geringsten.*

Erfolgt die Entnahme der Abstriche von einem anderen Ort, so muss der Cytologe darüber informiert werden, um die möglicherweise dadurch bedingten Unterschiede im Zellbild berücksichtigen zu können.

*Abstriche vom äusseren Drittel der Vagina zeigen bei Frauen mit atrophischem Epithel eine höhere Proliferation, während die Reaktion nach Follikelhormongabe an dieser Stelle verhältnismässig träge ist.*

Umgekehrt *lassen Abstriche von der Portio bei Frauen im geschlechtsreifen Alter oder nach künstlicher Hormonzufuhr oft eine etwas erhöhte Proliferation sowie eine geringere Faltung und Haufenbildung der Zellen erkennen. Die Abstriche erwecken dadurch häufig den Eindruck einer höheren Follikel-Hormon-wirkung bzw. eines geringeren Corpus-luteum-Hormonein-flusses als die Zellbilder vom seitlichen Scheidengewölbe.*

Soweit Abstriche aus dem Cervicalkanal wegen ihres reichlichen Gehalts an Schleim und Zylinderzellen für die hormonale Diagnostik überhaupt brauchbar sind, zeigen sie die Eigenarten des Portioabstrichs in noch deutlicherer Form.

Die Abstriche aus dem hinteren Scheidengewölbe als Sammeltopf für Zellen verschiedener Regionen spiegeln im allgemeinen Ubergänge zwischen den Unterschieden an den einzelnen Entnahmestellen wieder.

*Mischbilder mit Zellen aus allen Schichten des Epithels können durch Verletzungen der obersten Epithelschichten, durch Einschleppung von Zellen aus der Gegend des Introitus sowie durch Hormonmangelzustände verschiedenen Grades entstehen.* Zur Klärung der Ursache führt meist die Entnahme mehrerer Abstriche von verschiedenen Stellen.

Es mag auf diese Feinheiten nicht in allen Fällen ankommen. Eine grobe Orientierung über die Hormonlage ist bei Entnahme von fast jedem Bereich der Vagina möglich. *Für eine genauere hormonale Diagnostik* mit Berücksichtigung verschiedener Grade der Follikelhormonwirkung, von Corpusluteum-Hormon-und Androgen-Einflüssen aber *erscheinen uns eine exakte Entnahmetechnik und die Beachtung der Unterschiede im Zellbild unerlässlich, die sich durch die Entnahme der Abstriche von verschiedenen Stellen ergeben können.*

## SUMARIO

Basados en estudios comparativos hechos sobre 250 mujeres se prueban diferencias definitivas en los cuadros citologicos en variadas muestras vaginales y ectocervicales. Se discute el significado de estos hallazgos para la valoración hormonal del paciente por medio del examen citologico.

La zona de elección para el preparo de los extendidos citologicos para valoración hormonal de la mujer es le tercio projimal de la pared lateral de la vagina. En este sitio los cuadros citologicos corresponden aproximadamente a las condiciones hormonales verdaderas, como también alli los factores perturbadores non hormonales son minimos.

En los casos donde el especime citologico es preparado de sitios otros que non la pared lateral de la vagina, el citólogo debe ser informado del hecho para ser llevado a considerar las diferencias en el cuadro celular resultantes probables de la alteración de tecnica.

Especimes preparados del tercio distal de la vagina señalan un alto grado de proliferación en mujeres con epitelio vaginal atrófico, mientras la respuesta a la ministración de hormonas es relativamente pobre en dicho sitio.

De modo inverso, en especimes ectocervicales de mujeres durante la edad reproductiva, después de la ministración de hormonas, con frecuencia se nota un alto grado de proliferación y menor grado de doblamiento y ayntamiento celular. Por eso los especimes ectocervicales pueden ofrecer la impressión de un efecto estrogenico u progestogenico menor que en los extendidos de la pared lateral de la vigina. Los especimes endocervicales muestran aspectos màs pronunciados que los ectocervicales por su contenido de muco y celulas glandulares. Los especimes sacados del fondo de saco posterior de la vagina, como pozo coleccionador de las celulas originadas de los distintos puntos, en general exhiben estadios transicionales tras las diferencias de las variadas zonas donde fué preparado el material.

Los cuadros citologicos constituidos de lelulas de las distintas camadas pueden ocurrir como resultado de laceraciones epiteliales o de contaminaciones con celulas del introitus vaginal, o como resultante de deficinecias hormonales de distintos grados. En casos de duda, la causa puede ser encontrada con el preparo de especimes de las distintas zonas.

Estos puntos relativamente delicados pueden no ofrecer importancia en cada caso de per se. Una orientación general comprendendo la condición hormonal basica es posible con

el examen citologico de casi qualquier punto del epitelio vaginal. Para una mas exata valoración hormonal, entretanto, en especial cuando se consideran los distintos grados de atividade estrogenica, progestogenica y androgenica, la tecnica correta de preparación es importante y parece indispensable la consideración de las diferencias en los cuadros celulares que pueden resultar de preparaciones con especimes de distintos sitios del conduto reproductor femenino.

## BIBLIOGRAPHY


1. Adler, K.: Arch. Gynäk. 134–504, 1928.
2. Arronet, G. H.: Obstetr. Gynec. 10:636, 1957.
3. Peters, H., Israel, S. and Purshottam, S.: Fertility & Sterility 9:134, 1958.
4. Peters, H.: Zbl. Gynäk. 80:1049, 1958.
5. Pundel, J. P.: Les frottis vaginaux endocriniens. Paris, 1952, Masson.
6. Pundel, J. P.: Acquisitions recentes en cytologie vaginale hormonale. Paris, 1957, Masson.
7. Schmitt, A.: Geburtsh. u. Frauenk. 13:593, 1953.
8. Smolka, H., and Soost, H. J.: Grundriss und Atlas der gynäkologischen Cytodiagnostik. Stuttgart, 1956, Thieme.
9. Soost, H. J.: Acta Cytol. 3:177, 1959.
10. Stieve, H.: Zbl. Gynäk. 55:194, 1931.
11. Wied, G. L.: Amer. J. Clin. Path. 25:742, 1955.

# THE SYMPOSIA BY CORRESPONDENCE OF ACTA CYTOLOGICA

## INTRODUCTORY REMARKS

The Symposia of ACTA CYTOLOGICA are *held entirely by correspondence* and contain international discussions of scientific problems of interest to the exfoliative cytologist.

*System for Selecting Subjects for Symposia:* From recommendations received, the Editorial Office will draw up the list of subjects and will publish these subjects in ACTA CYTOLOGICA, under the heading FUTURE SYMPOSIA.

*Instructions for Authors:* Each problem will be introduced by a *Main Speaker* or *Speakers*. These principal papers will then be considered by persons identified as *Discussants*. As a general rule, approximately 600 words each will be allocated for main papers and 200 words each will be allocated for the contributions of the Discussants. The Main Speakers will then be given the opportunity to make unlimited Closing Remarks.

Photomicrographs and tables may be reproduced: one full page for each principal paper and for the paper of the Discussant (maximum one-half page per contribution). The photomicrographs and tables should be submitted in glossy photographic prints, preferably in the size of 4 × 5 inches (i.e., 10 × 12 cm) and should show a proportional 10$\mu$ scale on its reverse side. *Each figure should be accompanied by a comprehensive caption.*

The Discussants are requested *to strictly restrict their contributions to the discussion of the main papers*. Discussions which are not directly related to the main paper cannot be accepted. It is suggested that the Discussants prepare their contributions in such a manner that the reader may gain the impression of an actual round table conference.

The Closing Remarks of the Main Speakers should be limited to the answering of questions raised in the discussion and to other directly related information.

The Bibliography for the papers of both Main Speaker and Discussant should be organized in the same manner as in the American Journal of Obstetrics and Gynecology, at the *end* of the paper. *Every cited opinion or publication should have a reference in the bibliography.*

*Deadline for Contributions:* The Editorial Office will set deadlines for each written symposium. These will include:

1. deadline for agreements to contribute.
2. deadline for main papers.
3. deadline for discussions.
4. deadline for closing remarks.

*Reprints:* Authors may receive reprints of their papers by ordering these reprints before the particular issue goes to press.

## LES SYMPOSIA PAR CORRESPONDANCE DES ACTA CYTOLOGICA

Les *Symposia par Correspondance* des ACTA CYTOLOGICA présentent des discussions internationales sur des problèmes scientifiques intéressant le cytologiste exfoliative.

*Système du choix des sujets pour les symposia:* En partant des propositions, et sous la rubrique: FUTURS SYMPOSIA, le bureau de rédaction dressera la liste des sujets principaux qui seront publiés dans les ACTA CYTOLOGICA.

*Recommandations pour les auteurs:* Chaque sujet principal sera présenté par un Rapporteur Général ou des Rapporteurs. Ces mémoires principaux seront alors soumis aux Participants à la Discussion. En règle générale 600 mots seront accordés aux Rapporteurs des sujets principaux, et, 200 mots aux Participants à la Discussion. Les Rapporteurs Généraux pourront clôturer les discussions par un nombre illimité de remarques.

Des microphotos et graphiques pourront être reproduits à raison d'une page entière pour chaque sujet principal et une demi page au maximum pour les discussions. Les microphotos et les graphiques doivent être présentés sur du papier brillant, de préference dans le format 10 × 12 cm. *Chaque figure devra être accompagnée d'une legende explicative précise.*

Les membres et invités prenant part aux discussions sont invités à *limiter strictement leurs interventions aux discussions des sujets principaux.* Des discussions qui n'ont pas de rapport direct avec le sujet principal *ne pourront être acceptées.* Il est recommandé que les discussions soient rédigées d'une manière telle que le lecteure aît l'impression d'assister à une discution réelle de table ronde.

Les Remarques de Clôture du Rapporteur Général devront se limiter à la réponse aux questions soulevées dans les discussions et aux autres informations éventuelles ayant un rapport direct avec le sujet.

La bibliographie des rapports et discussions devra être rédigée de la même manière que celle de l'American Journal of Obstetrics & Gynecology et figurer à *la fin* du texte. *Chaque opinion ou publication citée dans le texte doit avoir sa réference dans la bibliographie.*

*Dates limite pour les collaborations:* Le bureau de rédaction, fixera des dates limites comprenaet:

1. un délai pour l'acceptation des collaborations,
2. un délai pour les sujets principaux,
3. un délai pour les discussions,
4. un délai pour les remarques de clôture.

Tirés-à-part: les auteurs pourront obtenir des tirés-à-part de leurs communications en les demandant avant la mise sous presse des ACTA CYTOLOGICA publiant leurs articles.

## DIE SCHRIFTLICHEN SYMPOSIEN DER ACTA CYTOLOGICA

Die schriftlichen Symposien der ACTA CYTOLOGICA befassen sich auf internationaler Basis mit wissenschaftlichen Problemen, die für den Exfoliativ-Zytologen von Interesse sind.

*System der Thema-Auswahl für die Symposien:* Die Schriftleitung stellt auf Grund von Thema-Vorschlägen eine Liste von Haupt-Themen zusammen, und gibt diese Liste unter dem Titel ZUKÜNFTIGE SYMPOSIEN bekannt.

*Instruktionen fur Autoren:* Jedes Thema wird von einem oder mehreren Referenten behandelt. Diese Referate werden dann von Diskussions-Vortragenden besprochen. Im allgemeinen werden Referate auf etwa 600 Worte beschränkt, und Diskussions-Vorträge auf 200 Worte. Die Referenten erhalten dann die Gelegenheit, Schlussbemerkungen ohne Wortzahlbeschränkung zu machen.

Mikrophotographien und Tabellen können abgedruckt werden: eine Ganzseite kann Referenten und eine halbe Seite Diskussionsvortragenden für Abbildungen zur Verfügung gestellt werden. Die Photographien sind auf Hochglanzpapier, und möglichst in der Grösse 10 × 12 cm erbeten und soll ein proportionales 10$\mu$ Zeichen auf der Rückseite haben. *Jede Abbildung muss von einem erklärenden Untertitel begleitet sein.*

Die Diskussionsvortragenden sind gebeten, sich in ihren Beiträgen *streng an das Hauptreferat zu halten.* Diskussionsbeiträge, die sich nicht an das Hauptthema halten, *können nicht berücksichtigt werden.* Es wird vorgeschlagen, dass die Diskussionsvorträge in einem Stil abgefasst sind, dass der Leser den Eindruck gewinnt, als ob es sich um eine Diskussion am runden Tisch gehandelt hätte.

Die Schlussbemerkungen der Referenten sollen sich nach Möglichkeit auf die Beantwortung von Diskussionsfragen beschränken.

Die Bibliographie der Referate und der Diskussions-Vortäge sollen *am Schluss* der Beiträge nach dem Muster der Bibliographien im American Journal of Obstetrics and Gynecology aufgeführt werden. *Jede zitierte Ansicht oder Publikation muss eine Referenz in der Bibliographie haben.*

*Termine für Beiträge:* Die Schriftleitung setzt Termine für die Schriftlichen Symposien fest. Die folgenden Termine werden bekanntgegeben:

1. Termin für Erhalt der Beitrags-Zusagen,
2. Termin für Erhalt der Hauptreferate,
3. Termin für Erhalt der Diskussions-beiträge.
4. Termin für Erhalt der Schlussbemerkungen.

*Sonderdrucke:* Autoren können Sonderdrucke ihrer Beiträge bestellen, bevor die betreffende Ausgabe in Druck geht.

## SIMPOSIUM ESCRITO DE ACTA CYTOLOGICA

El simposium escrito de ACTA CYTOLOGICA contiene discusiones internacionales sobre problemas científicos que son de interés para el citólogo exfoliativo.

*Sistema de selección de materias para el simposium:* Con sugestiones recibidas, la oficina editorial confeccionará una lista de los temas más interesantes, lista que será publicada en ACTA CYTOLOGICA con dos numeros de anticipación a la fecha de su posible publicación, bajo el epígrafe de "SIMPOSIUM FUTUROS."

*Participación en el Simposium Escrito:* No habrá restricción alguna sobre el número de puntos de discusión en los que cualquier autor desee participar.

*Instrucciones a los Autores:* Cada problema deberá ser presentado por un ponente o ponentes. Estos trabajos principales serán entonces discutidos por los comunicantes. Como regla general, se permite un máximo de 600 palabras para los trabajos principales y 200 palabras para las contribuciones de los comunicantes. Al ponente principal se le da la oportunidad de hacer rectificaciones finales ilimitadas.

Pueden reproducirse microfotografías y tablas: una página por cada trabajo principal y un máximo de media página por discusion. Las microfotografías y tablas deberán enviarse en forma de copias fotográficas amplias. A ser posible de 4 × 5 pulgadas (10 × 12 cms). *Cada figura deberá acompañarse de su correspondiente leyenda.*

Se suplica a los comunicantes *ajustar estrictamente sus comunicaciones a la discusión de los trabajos principales.* Las discusiones que no estén directamente relacionadas con el trabajo principal *no podrán ser aceptadas.* Se sugiere que los comunicantes realicen sus contribuciones de manera tal que el lector tenga la impresión de estar ante una verdadera mesa redonda.

Las rectificaciones finales de los ponentes deberán limitarse a contestar las preguntas aparecidas a lo largo de la discusión asi como a otras directamente relacionadas con el tema.

La bibliografia, tanto de las ponencias como de las comunicaciones deberá redactarse de la misma forma que figura en el American Journal of Obstetrics and Gynecology, *al final* del trabajo. *Toda opinión o publicación citada deberá tener su correspondiente referencia en la bibliografía.*

Fechas para las contribuciones: La Oficina Editorial, fijará fechas límite absolutas para cada simposium escrito. Estas incluirán:

1°. Fecha límite para acuerdo de contribución,
2°. Fecha límite para las ponencias,
3°. Fecha límite para las discusiones,
4°. Fecha límite para las anotaciones finales.

*Reimpresiones:* Los autores pueden recibir reimpresiones de sus trabajos si las ordenan antes de owe cada numero vaya a la imprenta.

# PROGRAM OF SYMPOSIA OF VOL. IV, NOS. 2 & 3, 1960

## *Symposium A*

## ADVANTAGES AND DISADVANTAGES OF VARIOUS TECHNIQUES OF OBTAINING MATERIAL FOR ROUTINE CYTOLOGICAL EXAMINATION


Page

Review of Techniques of Vaginal Smears . . . . . 221

WARREN C. HUNTER, Portland, Oregon, U.S.A.
J. PAUL PUNDEL, Luxembourg, Luxembourg
ABRAHAM E. RAKOFF, Philadelphia, Pennsylvania, U.S.A.
HOWARD L. RICHARDSON, Seattle, Washington, U.S.A.

Disc: Werner Bickenbach and Hans-Jürgen Soost, Munich, Germany
Marcel Gaudefroy, Lille, Nord, France
Ruth M. Graham, Buffalo, New York, U.S.A.
Emmerich von Haam, Columbus, Ohio, U.S.A.
Pierre Haour, Lyon, Rhône, France
George H. Romberg, White Plains, New York, U.S.A.
Guillermo Terzano, Buenos Aires, Argentina

Review of Techniques of Cervical Smears . . . . . 226

HERBERT E. NIEBURGS, New York, New York, U.S.A.

Disc: Arturo Angel Arrighi, Buenos Aires, Argentina
Emmerich von Haam, Columbus, Ohio, U.S.A.
Warren R. Lang, Philadelphia, Pennsylvania, U.S.A.
Luis Montalvo-Ruiz, Madrid, Spain
Otakar Nyklíček, Náchod, Czechoslovakia
Howard L. Richardson, Seattle, Washington, U.S.A.
George H. Romberg, White Plains, New York, U.S.A.

Review of Techniques of Endocervical Smears . . . . . 229

HERBERT E. NIEBURGS, New York, New York, U.S.A.
HORST SMOLKA, Kiel, Germany

Disc: Emmerich von Haam, Columbus, Ohio, U.S.A.
Luis Montalvo-Ruiz, Madrid, Spain
Violette M. Nuovo, Paris, France
Abraham E. Rakoff, Philadelphia, Pennsylvania, U.S.A.
Howard L. Richardson, Seattle, Washington, U.S.A.
George H. Romberg, White Plains, New York, U.S.A.

Review of Techniques of Intrauterine Smears . . . . . 233

HERBERT E. NIEBURGS, New York, New York, U.S.A.
HOWARD L. RICHARDSON, Seattle, Washington, U.S.A.
GEORGE H. ROMBERG, White Plains, New York, U.S.A.

Page

Disc: Pierre Haour, Lyon, Rhône, France
Abraham E. Rakoff, Philadelphia, Pennsylvania, U.S.A.

Material Obtained by Pipette from Vaginal Pool Only, without Visualization of Uterine Cervix . . . . . 236

EMMERICH von HAAM and EDWARD M. MILLER, Columbus, Ohio, U.S.A.

Disc: Werner Bickenbach and Hans-Jürgen Soost, Munich, Germany
Marcel Gaudefroy, Lille, Nord, France
J. Paul Pundel, Luxembourg, Luxembourg

Material Obtained by Pipette from Vaginal Pool Only, after Insertion of Speculum . . . . . 238

LUIS MONTALVO-RUIZ, Madrid, Spain

Disc: Marcel Gaudefroy, Lille, Nord, France
J. Paul Pundel, Luxembourg, Luxembourg

Material Obtained by Vaginal Tampon Only . . . . . 240

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany
HERBERT E. NIEBURGS, New York, New York, U.S.A.

Disc: Genevieve Bader and Emerson Day, New York, New York, U.S.A.

Material Obtained by Cervical Scraping Only . . . . . 242

FRIEDRICH BAJARDI, Graz, Austria
HOWARD L. RICHARDSON, Seattle, Washington, U.S.A.

Disc: Clarice co Amaral Ferreira, Rio de Janeiro, Brazil
Pierre Haour, Lyon, Rhône, France
Warren C. Hunter, Portland, Oregon, U.S.A.
Alexander Meisels, México, D.F., Mexico
Luis Montalvo-Ruiz, Madrid, Spain
J. Paul Pundel, Luxembourg, Luxembourg

Material Obtained by Two Techniques: (a) Vaginal Smears and (b) Cervical Smears . . . . . 246

JAMES HENRY FERGUSON and MARTIN H. MATZ, Miami, Florida, U.S.A.
EMMERICH von HAAM and EDWARD M. MILLER, Columbus, Ohio, U.S.A.
JOSEPH S. SONG, Providence, Rhode Island, U.S.A.
STANLEY WAY and NANCY DAWSON, Newcastle-Upon-Tyne, England, U.K.

Disc: Ruth M. Graham, Buffalo, New York, U.S.A.
Pierre Haour, Lyon, Rhône, France
Alexander Meisels, México, D.F., Mexico
Luis Montalvo-Ruiz, Madrid, Spain
Hannah Peters, Copenhagen, Denmark
Wolfgang Walz, Heidenheim, Brenz, Germany

Material Obtained by Three Techniques: (a) Vaginal Smears, (b) Cervical Smears and (c) Endocervical Smears . . . . . 252

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany
JEAN A. de BRUX, Paris, France
EMMERICH von HAAM, Columbus, Ohio, U.S.A.
LUIS MONTALVO-RUIZ, Madrid, Spain

Disc: Friedrich Bajardi, Graz, Austria
Pierre Haour, Lyon, Rhône, France
Constantin Herovici, Paris-Villejuif, France
J. Paul Pundel, Luxembourg, Luxembourg

Material Obtained by Sponge Biopsy . . . . . 257

PETER STOLL, Heidelberg, Germany

Disc: Wolfgang Korte, Bonn, Germany

If One Prepares More Than One Smear per Patient, Could One Prepare These Smears on One Glass Slide or Does One Have to Put Them on Separate Glass Slides? . . . . . 260

GUNTER F. BAHR and GEORGE L. WIED, Chicago, Illinois, U.S.A.
JOSEPH S. SONG, Providence, Rhode Island, U.S.A.

Page

Disc: Werner Bickenbach and Hans-Jürgen Soost, Munich, Germany
Marcel Gaudefroy, Lille, Nord, France
Emmerich von Haam, Columbus, Ohio, U.S.A.
Wolfgang Korte, Bonn, Germany
Hannes Kremer, Vienna, Austria
Luis Montalvo-Ruiz, Madrid, Spain
Wolfgang Walz, Heidenheim, Brenz, Germany

In Case of a Suspicious Ectocervix, Should a Biopsy be Done without Cytology? . . . . . 264

FRIEDRICH BAJARDI, Graz, Austria
M. KAWECKA, J. BYLINA, I. HLINIAK, H. STARKIEWICH, Gliwice, Poland
HANNES KREMER, Vienna, Austria
JOSEPH S. SONG, Providence, Rhode Island, U.S.A.
PETER STOLL, Heidelberg, Germany

Disc: Werner Bickenbach and Hans-Jürgen Soost, Munich, Germany
Marcel Gaudefroy, Lille, Nord, France
B. Cornelis Hopman, Miami, Florida, U.S.A.
Wolfgang Korte, Bonn, Germany
Ernst-Helmut Krüger, Halle a.d. Saale, Germany
Warren R. Lang, Philadelphia, Pennsylvania, U.S.A.
J. Paul Pundel, Luxembourg, Luxembourg

A Comparison of Blind Aspiration of the Vaginal Fluid and Aspiration Under Visualization . . . . . 270

JEAN L. JOHNSON and TED LACH, Buffalo, New York, U.S.A.

## *Symposium B*

## ENDOCERVICAL ADENOCARCINOMA

Histomorphology of Endocervical Adenocarcinoma . . . . . 271

JEAN A. de BRUX and MICHELYNE ANCLA, Paris, France
EMMERICH von HAAM, Columbus, Ohio, U.S.A.
RAIMUND KRIMMENAU, Dresden, Germany

Disc: Constantin Herovici, Villejuif, Seine, France
F.A. Langley, Manchester, England, U.K.

Histochemistry of Endocervical Adenocarcinoma . . . . . 291

CONSTANTIN HEROVICI, Villejuif, Seine, France

Cytomorphology of the Normal Endocervix . . . . . 293

CARY M. DOUGHERTY, Baton Rouge, Louisiana, U.S.A.

Disc: Maria S. Blanco de del Campo, Palo Alto, California, U.S.A.
Clarice do Amaral Ferreira, Rio de Janeiro, Brazil
Emmerich von Haam, Columbus, Ohio, U.S.A.

Cytochemistry of Normal and Abnormal Endocervix . . . . . 297

CONSTANTIN HEROVICI, Villejuif, Seine, France

Disc: Alvan G. Foraker, Jacksonville, Florida, U.S.A.

Fluorescence Microscopy of Endocervical Cells and Macrophoges . . . . . 298

LUDWIG von BERTALANFFY and FELIX BERTALANFFY, Topeka Kansas, U.S.A.
and Winnipeg, Canada

Differentiation of Endocervical Glandular and Metaplastic Cells . . . . . 299

JEAN A. de BRUX and J. DUPRÉ-FROMENT, Paris, France

Disc: Raimund Krimminau, Dresden, Germany
Alexander Meisels, México, D.F., México

Page

Histiocytes and Endocervical Cytology . . . . . 304

MARCEL GAUDEFROY, Lille, Nord, France

Disc: Maria S. Blanco de del Campo, Palo Alto, California, U.S.A.
Emmerich von Haam, Columbus, Ohio, U.S.A.
Alexander Meisels, México, D.F., México

Cytology of Endocervicitis . . . . . 306

MARCEL GAUDEFROY, Lille, Nord, France
EMMERICH von HAAM, Columbus, Ohio, U.S.A.
RAIMUND KRIMMENAU, Dresden, Germany
GUILLERMO TERZANO, Buenos Aires, Argentina

Disc: Clarice do Amaral Ferreira, Rio de Janeiro, Brazil
Warren R. Lang, Philadelphia, Pennsylvania, U.S.A.
Alexander Meisels, México, D.F., México
Luis Montalvo-Ruiz, Madrid, Spain
Erica Wachtel, London, England, U.K.

Cytology of Endocervical Hyperplasia and Reserve Cell Hyperplasia . . . . . 311

ARTURO ANGEL ARRIGHI, Buenos Aires, Argentina
JEAN A. de BRUX and J. DUPRÉ-FROMENT, Paris, France

Disc: Maria S. Blanco de del Campo, Palo Alto, California, U.S.A.
Alexander Meisels, México, D.F., México

Cytology of Endocervical Polyps . . . . . 319

JEAN A. de BRUX and J. DUPRÉ-FROMENT, Paris, France

Disc: Marcel Gaudefroy, Lille, Nord, France
Leopold G. Koss, New York, New York, U.S.A.
Warren R. Lang, Philadelphia, Pennsylvania, U.S.A.
Luis Montalvo-Ruiz, Madrid, Spain
Guillermo Terzano, Buenos Aires, Argentina

Cytology of Endocervical Adenocarcinoma . . . . . 323

JEAN A. de BRUX and J. DUPRÉ-FROMENT, Paris, France
B. CORNELIS HOPMAN, Miami, Florida, U.S.A.
VIOLETTE M. NUOVO, Paris, France

Disc: Emmerich von Haam, Columbus, Ohio, U.S.A.
Olle Kjellgren, Gothenburg, Sweden
Raimund Krimmenau, Dresden, Germany
Alexander Meisels, México, D.F., México
Guillermo Terzano, Buenos Aires, Argentina
Erica Wachtel, London, England, U.K.

Can One Distinguish by Means of Cytology the Endocervical and Endometrial Adenocarcinoma? . . . . . 338

B. CORNELIS HOPMAN, Miami, Florida, U.S.A.
VIOLETTE M. NUOVO, Paris, France

Disc: Jean A. de Brux, Paris, France
Alexander Meisels, México, D.F., Mexico
Guillermo Terzano, Buenos Aires, Argentina
Erica Wachtel, London, England, U.K.

Incidence and Clinical Course of Endocervical Adenocarcinoma . . . . . 341

DIETRICH BUTTENBERG and PETER STOLL, Heidelberg, Germany

Cyto- and Histomorphology of Carcinoma of the Gartnerian Duct . . . . . 344

DIETRICH BUTTENBERG and PETER STOLL, Heidelberg, Germany

# *Symposium C*

## TRAINING OF THE CYTOTECHNOLOGIST


Page

Educational Pre-requisites for Cytotechnologists . . . . . 347

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany
BIRGITTA FREDRICKSON, Gothenburg, Sweden
EDMUND SCHÜLLER and MARGARETE PEUTSCH, Vienna, Austria

Disc: Rachel R. Achenbach, Salem, Massachusetts, U.S.A.
Maria S. Blanco de del Campo, Palo Alto, California, U.S.A.
Alice Dargan Clark, Chicago, Illinois, U.S.A.
Elizabeth H. Gray, Ottawa, Ontario, Canada
Patricia Kelly, San Francisco, California, U.S.A.
Irena Koprowska, Philadelphia, Pennsylvania, U.S.A.
Raimund Krimmenau, Dresden, Germany
José R. del Sol, Madrid, Spain
Charlotte M. Street, New York, New York, U.S.A.
Dorothy B. Weller, Providence, Rhode Island, U.S.A.

Should Cytotechnology be Taught in Centralized Schools or in Individual Laboratories? . . . . . 351

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany
JEAN A. de BRUX, Paris, France
MARIA S. BLANCO de del CAMPO, Palo Alto, California, U.S.A.
EDMUND SCHÜLLER and MARGARETE PEUTSCH, Vienna, Austria

Disc: Rachel R. Achenbach, Salem, Massachusetts, U.S.A.
J. Ernest Ayre, Miami, Florida, U.S.A.
Birgitta Fredrickson, Gothenburg, Sweden
Marcel Gaudefroy, Lille, Nord, France
Elizabeth H. Gray, Ottawa, Ontario, Canada
H. Husslein, Vienna, Austria
Patricia Kelly, San Francisco, California, U.S.A.
Raimund Krimmenau, Dresden, Germany
Luis Montalvo-Ruiz, Madrid, Spain
Hannah Peters, Copenhagen, Denmark
Charlotte M. Street, New York, New York, U.S.A.
Pasco R. del Vecchio, Bethesda, Maryland, U.S.A.

Which Books and Monographs are Suggested for the Cytotechnologist Prior to and During the Practical Training? . . . . . 354

MARIA S. BLANCO de del CAMPO, Palo Alto, California, U.S.A.
BIRGITTA FREDRIKSON, Gothenburg, Sweden
WARREN R. LANG, Philadelphia, Pennsylvania, U.S.A.

Disc: Jean A. de Brux, Paris, France
Patricia Kelly, San Francisco, California, U.S.A.
Howard L. Richardson, Seattle, Washington, U.S.A.
Irena Koprowska, Philadelphia, Pennsylvania, U.S.A.

Program of Practical Training for Cytotechnologists . . . . . 357

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany
JEAN A. de BRUX, Paris, France
GRACE R. DURFEE, New York, New York, U.S.A.
EMMERICH von HAAM, Columbus, Ohio, U.S.A.
SARA HODGES, Charlotte, North Carolina, U.S.A.
PATRICIA KELLY, San Francisco, California, U.S.A.
PASCO R. del VECCHIO, Bethesda, Maryland, U.S.A.

Disc: Margaret E. Attwood, Birmingham, England, U.K.
J. Ernest Ayre, Miami, Florida, U.S.A.
Irena Koprowska, Philadelphia, Pennsylvania, U.S.A.
C. Pascal, N. Denavit and L. Troncy, Lyon, Rhône, France
Howard L. Richardson, Seattle, Washington, U.S.A.
Edmund Schüller, Vienna, Austria

Page

José R. del Sol, Madrid, Spain
Marvin W. Stafford, Philadelphia, Pennsylvania, U.S.A.
Charlotte M. Street, New York, New York, U.S.A.
Dorothy B. Weller, Providence, Rhode Island, U.S.A.

Program of Theoretical Education for Cytotechnologists . . . . . 366

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany
MARIA S. BLANCO de del CAMPO, Palo Alto, California, U.S.A.
GRACE R. DURFEE, New York, New York, U.S.A.
EDMUND SCHÜLLER and MARGARETE PEUTSCH, Vienna, Austria

Disc: Alice Dargan Clark, Chicago, Illinois, U.S.A.
Emmerich von Haam, Columbus, Ohio, U.S.A.
Irena Koprowska, Philadelphia, Pennsylvania, U.S.A.
Herbert E. Nieburgs, New York, New York, U.S.A.
C. Pascal, N. Denavit and L. Troncy, Lyon, Rhône, France
José R. del Sol, Madrid, Spain
Marvin W. Stafford, Philadelphia, Pennsylvania, U.S.A.
Charlotte M. Street, New York, New York, U.S.A.
Dorothy B. Weller, Providence, Rhode Island, U.S.A.

If there were a Licensing Examination for Cytotechnologists, of What Should This Examination Consist? . . 371

RACHEL R. ACHENBACH, Salem, Massachusetts, U.S.A.
CLAUD W. TAYLOR, Birmingham, England, U.K.

Disc: Pierre Haour, C. Pascal, N. Denavit and L. Troncy, Lyon, Rhône, France
Irena Koprowska, Philadelphia, Pennsylvania, U.S.A.
F.A. Langley, Manchester, England, U.K.
Charlotte M. Street, New York, New York, U.S.A.
José R. del Sol, Madrid, Spain

Should the Cytotechnologist be Trained to Take the Smears Himself? . . . . . 374

MARIA S. BLANCO de del CAMPO, Palo Alto, California, U.S.A.
BIRGITTA FREDRIKSON, Gothenburg, Sweden
RUTH M. GRAHAM, Buffalo, New York, U.S.A.

Disc: Werner Bickenbach and Hans-Jürgen Soost, Munich, Germany
Hanns-Werner Boschann, Berlin, Germany
Alice Dargan Clark, Chicago, Illinois, U.S.A.
Marcel Gaudefroy, Lille, Nord, France
Hannah Peters, Copenhagan, Denmark
Luis-Montalvo-Ruiz, Madrid, Spain
Peter Stoll, Heidelberg, Germany
Charlotte M. Street, New York, New York, U.S.A.
Olaf T. Messelt, Oslo, Norway

Should the Cytotechnologist be Trained in Hormonal and Microbiological Evaluation of the Vaginal Smears? . . . . . 377

JEAN A. de BRUX, Paris, France
BIRGITTA FREDRIKSON, Gothenburg, Sweden
EMMERICH von HAAM, Columbus, Ohio, U.S.A.
PATRICIA KELLY, San Francisco, California, U.S.A.

Disc: Rachel R. Achenbach, Salem, Massachusetts, U.S.A.
J. Ernest Ayre, Miami, Florida, U.S.A.
Werner Bickenbach and Hans-Jürgen Soost, Munich, Germany
Hanns-Werner Boschann, Berlin, Germany
Alice Dargan Clark, Chicago, Illinois, U.S.A.
Luis-Montalvo-Ruiz, Madrid, Spain
José R. del Sol, Madrid, Spain

What is the Average Screening Time for Smears from the Female Genital Tract, the Respiratory Tract, the Gastrointestinal Tract and the Urinary Tract? . . . . . 380

MARIA S. BLANCO de del CAMPO, Palo Alto, California, U.S.A.
BIRGITTA FREDRIKSON, Gothenburg, Sweden
HOWARD L. RICHARDSON, Seattle, Washington, U.S.A.

Disc: Arturo Angel Arrighi and Guillermo Terzano, Buenos Aires, Argentina
J. Ernest Ayre, Miami, Florida, U.S.A.
Ruth M. Graham, Buffalo, New York, U.S.A.
Elizabeth H. Gray, Ottawa, Ontario, Canada

Page

Emmerich von Haam, Columbus, Ohio, U.S.A.
Patricia Kelly, San Francisco, California, U.S.A.
Olaf T. Messelt, Oslo, Norway

What is the Average Capacity as to the Number of Cases of Cytological Smears per Day a Cytotechnologist Can Either Handle, Process and Screen or Screen Only? . . . . . 383

BIRGITTA FREDRIKSON, Gothenburg, Sweden
COLETTE PASCAL, NICOLE DENAVIT and LUCIENNE TRONCY, Lyone, Rhône, France
HOWARD L. RICHARDSON, Seattle, Washington, U.S.A.
EDMUND SCHÜLLER and MARGARETE PEUTSCH, Vienna, Austria

Disc: Ludwig von Bertalanffy and Felix D. Bertalanffy, Topeka, Kansas, U.S.A. and Winnipeg, Manitoba, Canada
Maria S. Blanco de del Campo, Palo Alto, California, U.S.A.
Ruth M. Graham, Buffalo, New York, U.S.A.
Elizabeth H. Gray, Ottawa, Ontario, Canada
Emmerich von Haam, Columbus, Ohio, U.S.A.
Irena Koprowska, Prima Baskelis and A. Darnell Corsey, Philadelphia, Pennsylvania, U.S.A.
Olaf T. Messelt, Oslo, Norway
José R. del Sol, Madrid, Spain
Dorothy B. Weller, Providence, Rhode Island, U.S.A.

Should the Cytotechnologist be Organized in His Own Organization? . . . . . 387

PIERRE HAOUR, COLETTE PASCAL, NICOLE DENAVIT and LUCIENNE TRONCY, Lyon, Rhône, France
EDMUND SCHÜLLER and MARGARETE PEUTSCH, Vienna, Austria

Disc: Maria S. Blanco de del Campo, Palo Alto, California, U.S.A.
Ruth Gibbert, Munich, Germany
Irena Koprowska, Philadelphia, Pennsylvania, U.S.A.
F.A. Langley, Manchester, England, U.K.
Pasco R. del Vecchio, Bethesda, Maryland, U.S.A.

Should a Certain Period of Training the Cytotechnologist be Devoted to Work in the Patient's Care? If so, Which Fields and Which Activities Should be Emphasized? . . . . . 389

BIRGITTA FREDRIKSON, Gothenburg, Sweden
RUTH GIBBERT, Munich, Germany

Disc: Maria S. Blanco de del Campo, Palo Alto, California, U.S.A.
Hannah Peters, Copenhagen, Denmark
Charlotte M. Street, New York, New York, U.S.A.

What is the Optimum Length of Time for Training? . . . . . 391

J. ERNEST AYRE, Miami, Florida, U.S.A.
JEAN A. de BRUX, Paris, France
JOSÉ R. del SOL, Madrid, Spain

Disc: Maria S. Blanco de del Campo, Palo Alto, California, U.S.A.
Birgitta Fredrikson, Gothenburg, Sweden
Elizabeth H. Gray, Ottawa, Ontario, Canada
Patricia Kelly, San Francisco, California, U.S.A.
Irena Koprowska, Philadelphia, Pennsylvania, U.S.A.
C. Pascal, N. Denavit and L. Troncy, Lyon, Rhône, France
Charlotte M. Street, New York, New York, U.S.A.
Guillermo Terzano, Buenos Aires, Argentina
Dorothy B. Weller, Providence, Rhode Island, U.S.A.

Should the Technician be Trained in All Aspects of Diagnostic Cytology, i.e., Sputum, Bronchial Aspiration, Urine, Gastric Secretion, etc.? . . . . . 393

RUTH M. GRAHAM, Buffalo, New York, U.S.A.
EMMERICH von HAAM, Columbus, Ohio, U.S.A.

Disc: Rachel R. Achenbach, Salem, Massachusetts, U.S.A.
J. Ernest Ayre, Miami, Florida, U.S.A.
Elizabeth H. Gray, Ottawa, Ontario, Canada
Irena Koprowska, Philadelphia, Pennsylvania, U.S.A.
Hannah Peters, Copenhagen, Denmark
Howard L. Richardson, Seattle, Washington, U.S.A.
Charlotte M. Street, New York, New York, U.S.A.
Guillermo Terzano, Buenos Aires, Argentina
Dorothy B. Weller, Providence, Rhode Island, U.S.A.

*Symposium A*

# ADVANTAGES AND DISADVANTAGES OF VARIOUS TECHNIQUES OF OBTAINING MATERIAL FOR ROUTINE CYTOLOGICAL EXAMINATIONS

## REVIEW OF TECHNIQUES OF VAGINAL SMEARS

WARREN C. HUNTER
Portland, Oregon, U.S.A.

The bivalve speculum constitutes a ready, simple and effective means for collecting fluid from the posterior fornix of the vagina. This technique of obtaining secretion for making smears has been used continuously in our clinics and hospitals since 1946 and has proved eminently satisfactory. The steps in the procedure are as follows:

1. Do not use a lubricant of any kind on the speculum.
2. Warm the instrument to the point of comfort, allowing all water to run off before insertion into vagina.
3. Using the posterior blade, gently scrape out such fluid as exists in the posterior fornix.
4. With a gloved fifth finger (dry but not covered by glove powder) dip enough fluid to cover the distal phalanx.
5. Starting three-fourths of an inch from one end of a new and clean glass slide, quickly and lightly move the finger to the opposite end of the slide; usually there is enough fluid to enable one to make a second parallel swipe. Commonly one thick ridge will occur at points of overlapping but otherwise the smears tend to be quite even and of good quality, without any more "bunching" of cells or great elongation of leukocytes than occurs when smears are made by the Papanicolaou method.
6. The technique is not recommended for obtaining material from the external os of the cervix.

J. PAUL PUNDEL
Luxembourg, Luxembourg

In his original technique for preparation of vaginal smears, Papanicolaou (1943) used a glass pipette attached to a rubber bulb, permitting aspiration of the vaginal content and its spreading upon a slide. This aspiration technique is apparently very easy and has been recommended by some authors for the use of the patient who can take daily vaginal smears at home.

In addition to the Papanicolaou pipette, other instruments have been proposed for the collection of cell material: a cotton swab (Schuman, 1944), a bacteriological wire loop (Rubenstein and Guterman, 1945), the valve of a speculum (Hunter and Richardson, 1947) and a spatula (Ayre, 1947; Pundel, 1950, 1952).

The instruments are introduced either directly into the vagina (blind collection) or the material is collected through a vaginal speculum, so that the site of collection is under visual control.

### Comparison of the Different Techniques

The choice of a collection technique depends first upon the purpose of the cytological examination. The first prerequisite for a good cytological diagnosis is to collect the material from the correct site and to

avoid unnecessary contaminations from other sources. For these reasons, vaginal smears are used mostly for hormonal diagnosis, while smears from the cervix are preferred for cancer detection. Therefore, I would like to discuss here only the choice of technique for hormonal vaginal smears.

Comparative studies have shown that the upper third of the vagina is the most sensitive area for hormonal stimulation while the squamous epithelium of the anterior third of the vagina and of the cervix is markedly less sensitive. Furthermore, it has been proved that the lateral side of a lateral vaginal cul de sac offers the best material for hormonal smears, not only because it offers the best hormonal reactions, but also because this part is exposed to a minimum of non-hormonal factors. Smears collected from the posterior fornix of the vagina are less appropriate because contamination of the vaginal cells by the cervical mucus can not be completely avoided. Needless to say, vaginal smears should be collected before any gynecological examination.

In order to obtain a correct collection of cytological material, it is necessary that it be done under visual control. A second important principle for the collection of vaginal smears is, therefore, the collection of the material under visual control through a vaginal speculum from an adequate site. Blind collection of smears does not permit one to obtain correct and selected cell material because the pipette can aspirate either only cervical mucus or a mixture of cervical mucus, vaginal cells and even cells from the vaginal orifice. The collection of the smears by the patient herself, possible only by blind aspiration, has been abandoned in our laboratory because the theoretical advantage of obtaining daily smears is neutralized by the bad quality of these smears.

Blind collection of hormonal smears is permitted only if the introduction of even a small speculum is not possible, such as in virgins with a small hymenal opening. Only in these cases do I accept blind collection of smears, but care has to be taken that the material is really collected from a lateral cul de sac of the vagina. For such blind collection one can use the usual pipette, but if a cotton swab or a bacteriological wire loop is used, one should insert it through a glass or paper tube, as proposed by Rubenstein and Guterman, in order to avoid contamination with vulvar or vaginal cells.

For the collection of vaginal smears through a speculum, all of the mentioned instruments can be used for the collection of material, but I personally prefer a metal or wooden spatula, either the special spatula described by Ayre or a common tongue blade spatula. The vaginal pipette permits a good collection of material only if the vaginal content is abundant. In older patients, where the vaginal content is scanty, it is sometimes impossible to obtain any material by aspiration, or, if after several aspirations, one has collected some material in the pipette, it is very difficult to get it out of the glass tube. The cotton swab permits a rather good collection, but the deposition of the material on the slide is sometimes difficult, because the dry cotton can absorb the vaginal cells, especially if the vaginal content is scanty. This can be avoided by moistening the cotton in normal saline solution before taking the smear.

The spatula permits a correct and sufficient collection of cell material in all cases, even if the vaginal content is scanty. If one uses a metal spatula, one does not need to moisten it as with a wooden spatula in the case of a "dry" vagina. The spatula takes off not only the cells contained in the vaginal pool, as does the pipette, but also the more superficial cells directly from the vaginal epithelium. The material obtained by the spatula is, therefore, in my opinion, the most adequate for hormonal diagnosis.

For the correct cytological diagnosis it is not only necessary that the smear is collected from the correct site in the vagina, but also that the material is correctly deposited on the slide with a minimum of mechanical alteration to the cells. The advantage of the spatula is, furthermore, that it permits a very regular spreading of the material on the slide in one stroke, without rotating movements so that the cell clusters are not destroyed and that all smears are very uniform.

As for cancer detection, the cytological test should never be used alone, but should be an obligatory part of a complete gynecological examination. I cannot see any particular advantage to a blind collection of smears for cancer detection. The vaginal smear should be taken for this purpose either directly from a suspicious lesion of the vaginal epithelium or from the posterior fornix. For a direct collection from a visible lesion, the spatula seems to be more convenient than the pipette, but for a usual vaginal smear from the posterior fornix, pipette and spatula are both adequate. The collected material is, in most cases, a mixture of vaginal cells and cervical mucus. However, as in my experience, routine vaginal smears taken from the posterior fornix, have given a rather important number of false negative diagnoses, I prefer smears taken directly from the cervix for general cancer detection.

Bibliography


1. Hunter, W. C. and Richardson, H. L.: Surg., Gyn. & Obst. 85:275, 1947.
2. Papanicolaou, G. N. and Traut, H. F.: N. Y. State J. Med. 43:767, 1943.
3. Pundel, J. P.: Les frottis vaginaux et cervicaux. Paris, 1950, Masson.
4. Pundel, J. P.: Les frottis vaginaux endocriniens. Paris, 1952, Masson.
5. Rubenstein, B. B. and Guterman, H. S.: Am. J. Obst. & Gyn. 50:565, 1945.
6. Schuman, W.: Am. J. Obst. & Gyn. 47:808, 1945.



## ABRAHAM E. RAKOFF

Philadelphia, Pennsylvania, U.S.A.


The choice of technique for collection of vaginal smears is influenced by (1) the purpose of the smear, (2) the age of the patient, (3) local anatomical factors, (4) the personnel collecting the smear. Quite a variety of techniques are available, each of which has certain merits and limitations with which the physician should be familiar so that he may choose that method best suited for the particular patient, even though he may prefer one particular method as "routine."

### I. For cancer screening

If smears are to be taken for cancer diagnoses, then collection of material from the vaginal pool in the posterior fornix is preferred, because this is the repository for cells from the entire genital tract. It is of course important for this purpose that the patient should not douche or use intravaginal medicaments, including contraceptives, for at least 24 hours beforehand. I prefer to take smears after insertion of the unlubricated or water-moistened speculum, prior to intravaginal digital examination. If a collection of material is apparent in the posterior fornix, then material is aspirated with a glass or plastic pipette attached to a rubber bulb or syringe. The latter has the advantage of permitting aspiration into just the fore-part of the pipette, and also permits more accurate expulsion onto the slide. If there is very little material apparent in the posterior fornix, the secretion in the posterior vagina is collected on an applicator tipped with non-absorbent cotton. Alternate methods are the use of a wooden or preferably plastic spatula to scoop-up the scant secretion or the use of the posterior blade of the

bivalve speculum for this purpose. In other words, the method employed should be that which affords the best opportunity for obtaining material in the particular case, rather than rigid adherence to one method which may not be best suited for all cases.

The use of the speculum as a preliminary to smear collection is favored not only because it permits visualization of the site and the amount of secretion, but also because it encourages speculum examination of the cervix in all instances, a point of great importance in detection of all sorts of cervical as well as vaginal lesions. It also encourages the collection of additional smears from the cervix, the cervical canal or endometrial cavity. I limit the method of aspiration of vaginal smears without the use of a speculum to children and to patients in whom speculum insertion may be difficult because of a rigid intact hymen or stenosis of the vagina. It should be mentioned in passing, however, that even in these instances a small narrow speculum can often be introduced without difficulty or discomfort. In screening programs in which large numbers of smears are collected by non-physicians, the aspiration method is used by technicians or nurses trained for this purpose. I do not prefer this technique except when no other alternative is available.

II. For hormonal diagnosis and vaginal infections

Material for smears taken primarily for endocrine evaluation is best collected from the lateral vaginal wall, because it is not contaminated with cervical or uterine material; it provides "fresh" cells which are more indicative of the hormonal effect of the day on which the smear was taken than are cells already exfoliated, and there is less degeneration, cytolysis, and bacterial and chemical action to interfere with staining reactions. Smears are best collected after speculum insertion from the mid-lateral vaginal wall on an applicator tipped with non-absorbent cotton and rolled over a glass slip and then fixed immediately. The introduction of the speculum also provides an opportunity to aspirate cervical mucus to be used for the fern reaction. Another method which we use extensively for office and clinic is to collect secretions in the same manner with the usual absorbent cotton-tipped applicator, which is then immediately dropped into a small test tube containing 2 cc of saline. A drop of this material is then transferred to a cover slip and examined, particularly for mobile trichomonads, and also for hyphae, yeast cells and leukocytes. One then adds cytoplasmic stains to the tube for rapid determination of estrogen effect on the wet smear.

We have found that the addition of three drops of the following staining mixture is very satisfactory for this purpose:

| | | |
|---|---|---|
| Light Green | 5% | 83 cc |
| Eosin Y | 1% | 17 cc |

After adding the stain, the mixture is agitated with the applicator and a drop transferred to a slide, covered with a slip and examined under the microscope for cellular morphology and the Eosinophilic Index. The nucleus is sufficiently outlined so that with proper adjustment of the light the Karyopyknotic Index can also be determined.

I quite routinely collect a wet smear preparation in all patients, even those in whom permanent slides for screening purposes are taken, because of the rapid initial evaluation which it affords with regard to hormonal function, infection, and sometimes presence of abnormal cells.

## HOWARD L. RICHARDSON

### Seattle, Washington, U.S.A.

Aspiration of the secretions from the vagina by means of a glass pipette, in my opinion, is not a good method for the diagnosis of cancer. If the method is to be successful it requires controlled conditions and is therefore only applicable to hospitals and staffs trained in its use.

Vaginal secretions, blood, mucus and even spermatic fluid may reduce the concentration of cancer cells on smears. The amount of time needed by cytotechnologists for screening these smears thus increases, making it impractical for routine use because of the expense involved.

In a routine examination, visualization of the vaginal vault and cervix is of extreme importance. Smears obtained by the introduction of a glass tube into the vagina may lead to a sense of false security.

Aspirations of vaginal fluid following a douche is of little value cytologically. In our experience, the majority of women visiting a clinic or physician's office, having pride in personal cleanliness, douche before their appointment.

The only advantage of this technique is in the study of the ovulatory cycle and the influence of the endocrine system on the epithelium.

## DISCUSSION

WERNER BICKENBACH and HANS-JURGEN SOOST, Munich, Germany:

The subject was explained extensively by the speakers, so there is nothing to be added. The technique of taking a smear, as it is used in our hospital, corresponds to the one described by Pundel. The experiences of Rakoff conform with it in many respects. However, he is very much in favor of the physician choosing the method of taking the smear according to the given circumstances. We think it adequate when smears are taken by someone who is familiar with the method. We think it useful to give physicians having less experience in this special field—as is the case for a large number of gynecologists and most practitioners and medical men—simple and clear directions which are strictly enforced. Otherwise the method of taking smears will become confused and the diagnosis will be uncertain. In accordance with Richardson we use the "blind" aspiration technique, without visualizing the cervix, only rarely. (See topic, Material Obtained by Pipette from Vaginal Pool Only, Without Visualization of Uterine Cervix). We do not take a smear with a speculum as recommended by Hunter, because a removal of material from the ectocervix in this circumstance is hardly possible. We have the same argument as Pundel against taking smears from the posterior vaginal fornix.

MARCEL GAUDEFROY, Lille, Nord, France:

There is almost nothing to add: it is satisfying that the routine introduction of a little bivalve speculum without lubrication has obtained a general agreement. Like Pundel and others, I have recommended that technique for ten years. In addition, I would like to point out an interesting fact. In smears prepared with material from a pipette only, false eosinophilic cells (irregularly colored in red or orange-yellow) are often observed without nuclear pyknosis. For a while I thought the drying of the smears was the reason for the false eosinophilia, but later while performing systematic smears of different parts of the vagina, from posterior cul-de-sac to vulva, I met the same type of false eosinophilic cells in smears from the vestibule, in the area of the periuretral and perihymeneal zone, where the epithelium is more keratinized than the vaginal mucosa and less than the skin. These false eosinophilic aspects make the smears unfit for reliable hormonal diagnosis.

RUTH M. GRAHAM, Buffalo, New York, U.S.A.:

There seems to be a general agreement that collection of cells from the vaginal mucosa is preferred for hormone evaluation. I would like to ask Pundel one question. He states that he prefers the metal spatula as the instrument to obtain material. It has been my understanding that hormonal evaluation depends on the maturation of the cells at the surface of the epithelium. Is it not possible that by scraping, deeper cells may be obtained whose exact position in the epithelium is unknown?

I agree that the advantage of a cervical scraping rather than a vaginal smear is that the cervix is visualized. However, this is a valuable by-product of cytology and not strictly speaking concerned with evaluation of methods of collection.

One difficulty here I think is that we may not be talking about the same kind of problem. Are we considering the application of cytologic diagnosis to large scale screening—tens of thousands of well people—or are we considering the patient who comes to the doctor's office or clinic with specific problems? Detection of early cancer may be accomplished by routine physical examination, as shown by MacFarlane (1). The advantage of cytology is that it can detect lesions which cannot be seen. If a lesion can be visualized it would seem more important to biopsy it than to scrape it.

There is a great deal said about the shortage of trained personnel in cytology. There is an even more acute shortage of trained physicians to examine well patients. After all visualization of the cervix is only of value if done by professional personnel.

May I again emphasize that the importance of the aspiration of the vaginal secretion is that it is representative of the entire epithelium. In 1948 at the First Cytology Conference sponsored by the American Cancer Society, Dr. William Boyd made a statement which is pertinent. "If cytologists receive more and more vigorous scrapings and the pathologists gentler and gentler biopsies, both will come to the same end." It is important that we do not lose the great advantage of cytologic diagnosis:

1. The detection of lesions which cannot be visualized.
2. The simplicity of obtaining the specimen.

Bibliography

1. MacFarlane, C.: J.A.M.A. 138:941, 1948.

EMMERICH von HAAM, Columbus, Ohio, U.S.A.:

All four essayists warn against the blind aspiration methods and prefer exposure of the vagina and cervix by a non-lubricated speculum regardless of the type of vaginal smear taken and the technique used. This of course excludes the preparation of vaginal smears by non-professional assistants or by the patients themselves. In our large survey of over 100,000 women in our community we convinced ourselves of the fact that the blind vaginal aspiration method is less accurate than the cervical scraping as far as cancer detection is concerned, while both techniques were sufficiently accurate for hormonal evaluation. However, we would not have discovered as many cases of symptomless cancers had we not employed the vaginal aspiration technique in order to examine as many women as possible in the available time. Outside of cancer surveys, we agree that the speculum should always be used to obtain reliable vaginal specimens.

PIERRE J. HAOUR, Lyon, Rhône, France:

I am of the opinion of Rakoff that the choice of a method of cell collection depends on the circumstances in which the smears are done. But among the different techniques which can be used , some give better results than others in cancer diagnosis or in the study of hormonal stimulus.

In cancer diagnosis, the aspiration with a glass pipette without visualization of the cervix is suitable in the hands of some cytologists, even for carcinoma of the endometrium. Nevertheless the general opinion is that this method does not give results as satisfactory as the cervical scraping for the detection of carcinoma of the cervix or the intrauterine aspiration for the detection of adenocarcinoma. However, there are some conditions in which it is not possible to use a speculum and the "vaginal aspiration" will be chosen. These conditions are anatomical (e.g., intact hymen or stenosis of the vagina after radium therapy) or practical (e.g., in a mass screening program or because of lack of trained medical staff).

In the study of hormonal conditions, I share Pundel's opinion that the best method is to collect cells on the lateral wall of the vagina under speculum visualization. We prefer the wooden spatula: with it the material collected is more easily spread. If one has time and wants better material the technique of the cotton swab previously dipped in saline solution is recommended.

Recently we began to try a "printing technique" which consists of applying the slide directly onto the vaginal wall. This procedure, which uses smaller slides, gives excellent cytological pictures of the cellular surface of the vagina. Small or large sheets of cells are obtained which are mostly made of juxtaposed cells, flat or folded cells according to the hormonal stimulus.

But when the smears cannot be performed under visualization with the speculum, aspiration in the vaginal pool with a pipette might be done. The material obtained by this technique is often mixed with cells exfoliated from the uterus which can also be inflammatory cells.

Another question, which was not mentioned by the main speakers, is the study of serial smears during the menstrual cycle. In this case, for practical reasons, the patient is asked to make the smears herself, using the vaginal pipette. Most of the time this "auto procedure" will not give satisfactory smears. Therefore, we prefer to use the urinary sediment technique, especially on young girls and in case of vaginal infection.

For studying inflammatory conditions the technique of Wied is simple and gives a precise interpretation.

GEORGE ROMBERG, White Plains, New York, U.S.A.:

It is interesting to note that the opinion of the four essayists regarding the value of vaginal smears for cancer and hormonal diagnosis reflects fairly well the general cross-section of medical opinion regarding vaginal cytologic smears.

Personally I favor the aspiration technique with visualization of the vaginal vault and cervix. Rarely was it found necessary to use a spatula.

GUILLERMO TERZANO, Buenos Aires, Argentina:

As Hunter, Pundel, Rakoff and Richardson have pointed out so well, the perfect procedure for obtaining specimens to study exfoliative cytology of the genital tract in women has not yet been developed. Therefore it is natural that preference may exist regarding the selection of one technique or another, such as aspirations, scrapings, tampons, diaphragms, Gladstone's sponges, Trifon's contact smears, wire loops, etc.

We agree that "the choice of collection technique depends upon the purpose of the cytological examination" and on "the purpose of the smear," because it is obvious that a single vaginal smear is not the same as serial vaginal smears; a Papanicolaou cytological test to rule out cancer for one patient is quite different from putting a mass screening project into action. All the factors have

to be detailed and discussed when related to special circumstances, for instance: the easiest way to obtain specimens, the quickest way to screen slides, time, price, etc. As a complement of the routine examination regularly performed at out-patient clinics, as "a part of the complete gynecological examination" which is the application we have given to the smear method here, we believe it is best to take the three classic smears recommended by Papanicolaou: a vaginal aspiration, a cervical scraping (cotton swab or wooden spatula) and an endocervical aspiration (endometrial aspiration being restricted to those cases in which bleeding, bloody discharge, etc., justifies going into the uterine cavity, or if it is requested by the patient's doctor).

In addition to endocrinological aspects of the method, it is interesting to keep a record of what has been found in vaginal aspirations: information on the trophic condition of the vaginal epithelium, the presence of unsuspected parasitic infection, unsuspected bleeding, etc.; apart from the fact of the vaginal pool being "the repository for cells from the entire genital tract," cancer cells shed off from the urethra can, for instance, be collected there.

For endocrinological evaluations, smears collected from the lateral vaginal wall are the most desirable material, but unfortunately we do not always have ideal conditions, and in practice, there are problems which must be solved.

There are cases in which daily smears are requested and the patient cannot be asked to come to the hospital every day for a vaginal aspiration. In these circumstances, "blind aspirations" are accepted. We may say that in our experience patients do it satisfactorily. Patients are asked to come to the hospital every four, five or seven days, so that a specimen may be obtained by us with the aid of a speculum. When smears are stained and studied all together, no remarkable differences are noticed between the smears prepared by the patient and those obtained by us. I personally prefer to accept blind smears than to have none.

We check our series periodically with urine sediment and when vaginal smears give suspicion of error, urocytograms cover the purpose satisfactorily.

I would like to emphasize again that exfoliative cytology is a very important tool in the armamentum of the modern gynecologist, but it does not destroy any of the established methods, which should not be underestimated. (We learned this too from Papanicolaou.) Clinical history of the patient, vaginal examination, visualization of the cervix, etc., are influential for gynecologists or general practitioners who are interested in detecting early cancer.

## CLOSING REMARKS

WARREN C. HUNTER:

I would like to thank Gaudefroy for his discussion. It is gratifying to learn that others have been using the speculum method of obtaining fluid from the posterior fornix and have found it satisfactory. Using this instrument insures seeing the cervix, which apparently is not true for those who use a tube and take the material blindly.

Since we never take smears by means of a pipette the observations regarding the presence of false eosinophilic cells has never been noted by us. This is an interesting finding.

J. PAUL PUNDEL:

It is very encouraging to see that the main speakers and discussants have developed the same basic opinions concerning the collection technique for cytological material. It seems now that blind preparation of specimens would be acceptable only for such cases where a visualization of the ectocervix is not possible either due to technical or anatomical conditions. If the specimen has to be prepared by a doctor, it should always be collected under visualization of the ectocervix and the choice of the site from which it should be taken depends upon the purpose of the cytological examination. Therefore, one can accept blind aspiration smears in mass screenings of large populations, as presented by von Haam, only if the smears have to be collected by technicians and if otherwise such a screening, even with its reduced results, would not have been possible. However, for blind preparation of the specimens by the patient in order to obtain daily specimens for hormonal studies, I have had the same experience as Gaudefroy and Haour, and I, therefore, prefer the same technique as recommended by Terzano. If it would not be possible for the patients to come to the hospital at least every four days for a correct preparation of the specimens, then I prefer daily urocytograms, provided the patient can send the urine specimens to the laboratory without delay.

To Ruth Graham: I would like to answer that the preparation of specimens for hormonal evaluation by the spatula is not really a "scraping" technique. The spatula slides in a very gentle manner over the vaginal epithelium so that it collects only the cells adherent to the wall, similar to aspiration by a pipette. The advantage of this technique is given by the ease in which the material can be deposited upon the glass slide. For cancer detection, I agree completely with Ruth Graham that the main purpose of cytology is to detect cancers which cannot be discerned by other techniques. If the lesion can be visualized, it should be studied by all available techniques. I also prefer the immediate biopsy, but I would also take a smear, because the biopsy might (1) be taken at the wrong area of (2) the obvious lesion can be entirely benign and a cancer can be concealed behind this area.

For the remaining minor differences, I think that they are only the result of different working conditions. We should not try to say that only one collection technique is the right one. It would be natural that some cytologists or clinicians prefer a different technique for technical, practical or financial reasons, especially if their results are as excellent as those obtained by others with different techniques.

# REVIEW OF TECHNIQUES OF CERVICAL SMEARS

HERBERT E. NIEBURGS
New York, New York, U.S.A.

Cellular material is collected from the portio vaginalis of the cervix, possibly including the cervical os by a "cervical smear." The next topic in this Symposium deals with techniques of endocervical smears; therefore, only a few methods are worthy of description within the context of this presentation.

The tampon method, which under ideal circumstances might be considered a cervical smear, is also discussed under a different heading in this Symposium.

Several collection methods for cervical smear preparation, although not designed to enter the endocervical canal, result in smears which may contain endocervical material due to the fact that this has been aspirated from the cervical os or removed with the mucous plug by wiping the cervix over the area of the external os.

Cervical smears may be obtained through a glass cannula by means of aspiration with a rubber bulb. Such aspiration can be carried out either exclusively from the posterior fornix which contains the vaginal pool or by moving the cannula over the cervical area. With both methods, the use of a speculum is optional, although preferable.

The surface scraping methods belong to the category of cervical smears, although cervical material may be abundant in the smear if mucus was removed from the cervical os. For this purpose a specially designed wooden spatula, as recommended by Ayre, may be used. A scraping device which will enter the cervix at times more deeply may be improvised by simply cutting a wooden tongue depressor with a curved scissors.

The gelfoam method originally described by Gladstone consists of briskly rubbing a piece of 1 cm square #12 gelfoam across the surface of the cervix. Then, the gelgoam is placed in formalin for embedding in paraffin and cutting of sections. The advantages, however, of the cytologic method, that is of superior preservation and visibility of the cellular structure, is sacrificed.

A new technique for cervical smears has recently been described by Trifon. This entails the introduction of a glass slide, 1 x 1.875 in., attached to a slide carrier into the vagina up to the surface of the cervix. It is gently pressed against the cervix to make a contact smear. This method requires the use of a speculum.

A technique designed to remove cervical material from the cervix was recently described by the author. This is carried out by means of a slide incorporated in a tube applicator and may be used with or without a speculum. The withdrawal of an inner plunger through the outer tube forms a smear on a plastic slide. This is stained in the usual manner and permanently placed in an inverted position on a glass slide similar to a cover slip.

Reports on the quality of smears obtained by various methods and the degree of accuracy for each technique differ widely. It is not uncommon to find that each method when applied by different individuals varies slightly in some steps of the procedure: certain motions during sampling which are particular to the individual, the method of transfer of material to the glass slide, methods of fixation and whether or not the mucous plug was wiped off the cervix prior to the collection of the specimen, may considerably influence the results.

Many investigators are now in agreement that a large number of cervical carcinomas may be missed if material is not collected directly from the endocervix. I should like to recommend strongly that, whenever possible, a smear for cancer detection of the cervix should be taken preferably directly from the endocervix. A simplified device which, according to preliminary results, appears to be more satisfactory than the endocervical smear is now being investigated by this laboratory and will be reported on in a different presentation.

## DISCUSSION

**ARTURO ANGEL ARRIGHI**, Buenos Aires, Argentina:

We consider the review of Nieburgs very complete and objective, and we also agree with him about the importance endocervical collection has in the detection of cervical carcinoma.

**EMMERICH von HAAM**, Columbus, Ohio, U.S.A.:

I consider it an important part of the cervical smear to include material from the external cervical os and prefer the surface scraping method carried out with Ayre's spatula or a trimmed-down tongue depressor before any other. In my laboratory it gives the least number of false negatives in cervical lesions but is not quite accurate enough for the detection of endometrial carcinomas, for which other techniques should be employed.

**WARREN R. LANG**, Philadelphia, Pennsylvania, U.S.A.:

Dr. Nieburgs has reviewed the various techniques for obtaining cervical cells, both squamous and columnar. I believe he would agree, however, that the use of a speculum is mandatory if the physician desires

assurance that the material is truly and mainly cervical in origin. Otherwise, in multiparous patients the applicator may press against a fold of vaginal wall with marked retroflexion of the uterus. The cervix may be high and anterior, and the applicator may thus be directed into a capacious posterior fornix.

It is true that endocervical material may be found in cervical mucus. The one point to make is that all columnar cells do not necessarily arise from the endocervical canal. Some originate on the portio from a benign "erosion," which is most commonly an area covered with columnar epithelium. The best way to obtain endocervical elements is to aspirate the endocervix directly.

It is commonly stated that many squamous cell carcinomas of the cervix arise in the endocervix, yet the fact remains that most biopsies diagnosing preinvasive and early invasive carcinoma are portio biopsies. For this reason it is wise to combine a cervical scraping technique with endocervical aspiration rather than to rely on one method alone.

LUIS MONTALVO-RUIZ, Madrid, Spain:

We would like to add the following techniques which were not mentioned in the review by Nieburgs: the platinum loop recommended by Rauscher, the cotton swab advised by Pund and Nettles and the direct intravaginal slide collection recognized by Hyams.

The choice of the technique varies if the woman is a virgin or not. If she is not, the introduction of the speculum facilitates any technique. On the other hand, if she is a virgin, and the speculum cannot be used, the choice of technique is limited. Nevertheless, with the very small speculum we have been able to visualize the ectocervix and collect the specimen under visual control in some patients, but when it is not possible, we suggest the usage of the pipette, in spite of the fact that it is less accurate. If possible, the cervical mucus must be avoided because it interferes with the hormonal reading. In another section of this Symposium we have discussed the advantages and disadvantages of different techniques.

We agree with Nieburgs in regard to his statement that the results of each technique vary according to the individual usage and every author changes little technical details which can vary the results.

OTAKER NYKLÍČEK, Náchod, Czechoslovakia:

I take the liberty to add to the review of techniques of cervical smears, such as had been extensively described by Nieburgs, our new imprint technique, employed by us for obtaining the materials of the whole ectocervix.

This technique is effected by means of vaginal specula and the imprints are obtained on halves of standard slides (2.6 x 4.0 cm—the slides may, however, be even larger according to the dimensions of the portio vaginalis, up to 3.5 x 4 cm).

We have solved this by means of our conductor. It is based on the principle that the slide carrier may be tilted by 90°. By a simple passing of the end of the handle of the conductor the slide carrier is put into the longitudinal position with the handle of conductor, so that it may easily pass through the vaginal introitus. Then we relax

Fig. 2.

the pressure tilting the slide carrier by 90°, so that now the slide is ready in front of the area of portio vaginalis. Now we succeed by just a slight pressing in catching the epithelial covering of the whole uterine cervix. Then again by a pressure on the lower end of the handle the slide carrier is tilted by 90° and passes easily by the vaginal introitus.

Before introducing the slide we mark:

1. the order number,
2. a respective side: D - right, S - left,
3. and by a little cross we mark the middle of the slide, which we try to insert at the orifitium externum.

The advantages of this imprinting technique:

1. In a large majority of cases we succeed in catching the cells of the whole area of the portio vaginalis by a direct, primary, imprinting.
2. According to a possible finding of pathological cells on some certain place of the

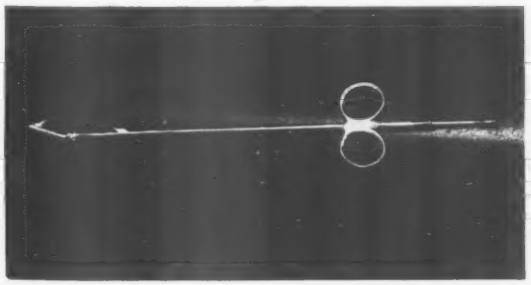


Fig. 1.

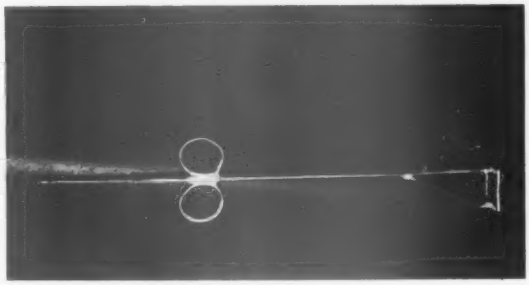


Fig. 3.

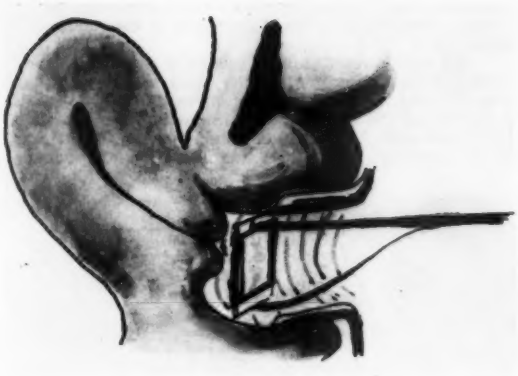


Fig. 4.

slide, one can retrospectively localize the site of the lesion on the ectocervix.

3. The cells are well imprinted and are not at all deformed.

H. L. RICHARDSON, Seattle, Washington, U.S.A.:

Dr. Nieburgs has reviewed the classical methods of obtaining cells from the cervix. As a discussant, I do not feel the gelfoam method described by Gladstone bears any further consideration in the field of cytology. It is a histological sectioning method. Contact smear techniques are to be avoided, for these methods may not get all areas of the cervix. In the opinion of the writer, any method advocated to circumvent visualization of the cervix is unsatisfactory. Into this category falls the aspiration techniques. In my own experience with individual physicians, the scrape smear techniques, as described by Ayre, are the most satisfactory for routine use. The circular motion advocated, on the portio vaginalis and endocervical canal, produces the highest degree of accuracy when a physician's office is a cancer detection center.

GEORGE ROMBERG, White Plains, New York, U.S.A.:

The only comment I would like to make is that I agree completely with Nieburg's statement that for cancer detection purposes, the endocervical smear is definitely preferable to the cervical one.

## NO CLOSING REMARKS

# REVIEW OF TECHNIQUES OF ENDOCERVICAL SMEARS

HERBERT E. NIEBURGS
New York, New York, U.S.A.

An endocervical smear is designed to remove cells from the endocervical canal as well as from the squamo-columnar junction at the external os. As such the endocervical smear should contain columnar cells from the endocervical canal and possibly also from the endocervical glands, as well as squamous cells originating from the site adjacent to the columnar epithelium at the external os. In addition, there is mucus and varying numbers of leukocytes. Unless such elements are present in the endocervical smear, a negative report should be given with certain reservations, since numerous authors have described 40-50% false negative smears when these were taken from sites other than the endocervix.

Only a few methods qualify under the heading of endocervical smears. The main procedures most widely used are aspiration from the cervical os by means of a rubber bulb through a glass or plastic cannula and removal of endocervical material by a cotton-tipped applicator. Both methods appear to be equally satisfactory with a similar degree of accuracy. Possibly a larger number of endometrial carcinomas may be detected by the method of aspiration, while the cotton-tipped applicator has the advantage of simplicity. In cases in which endometrial malignancy is suspected, the aspiration should be obtained directly from the endometrial cavity, in preference to the external os.

In a controlled comparative study, smears were taken from the cervical os with a cotton tipped applicator followed by scraping of the external os with Ayre's spatula. An additional third smear was obtained with a cotton applicator from the posterior fornix. From this study it appeared that the collection of the mucus plug from the cervical os constituted the most valuable aspect of the smear.

In two separate investigations, the smear taken from the posterior fornix from 28 women with known epithelial lesions resulted in 14 and 15 false negative smears. The comparative accuracy of smears made with either the cotton applicator or spatula depended on the order in which the smears were taken. False negative smears were obtained with each of the two methods when used as the second procedure.

It thus appears that the mucus plug serves as an ideal reservoir for malignant cells if present, and a second smear obtained immediately after removal of the mucus plug may not contain any malignant cells.

Endocervical smears obtained with a cotton-tipped applicator may yield unsatisfactory results if certain steps are not adhered to in the transfer of material to the slide. When the cotton-tipped applicator is rolled in its axis in the same direction in which it is guided over the slide, insufficient transfer of cells may occur due to the fact that the mucus has the tendency to stick to the cotton. If however, the cotton-tipped applicator is rolled in its axis in the direction opposite to that in which it is moved over the slide, a satisfactory transfer of mucus occurs.

Until recently, the immediate fixation of the smears in ether alcohol alone was an important factor. It was found, however, that smears from the cervical, endocervical and vaginal epithelium may be allowed to dry in air without impairment of visible cellular structure if in the laboratory the smears are subsequently reconstituted by a process of rehydration followed by fixation in alcohol before staining. Rehydration may be accomplished by using either Tween 80, sulfonated laurel or hydroxypropyl methyl cellulose ether, or any other agent which will decrease the surface tension of the water. The dry smear method has now been tested in my laboratory on more than 50,000 smears and appears to result in a lesser number of smears with effects of drying than those obtained supposedly by immediate fixation.

This is possibly due to the fact that in the physician's office often some drying may at times occur before fixation.

From experience gained during the last 16 years, it seems that at present the cotton-tipped applicator probably presents the method of choice for endocervical cell collection. Aspiration, however, should be carried out if the introduction of the cotton-tipped applicator is not possible due to either stenosis of the cervical os or inability to visualize the cervix.

HORST SMOLKA
Kiel, Germany

The following techniques are suitable for the procurement of cell material from the endocervical canal:

1) Aspiration of the endocervical mucus with its exfoliative cellular content by means of a glass pipette, similar to that devised by Papanicolaou, the forepart of which shows a particularly thin caliber. This method has the disadvantage that it is difficult to remove small quantities of material from within the

narrow glass tube.

2) Aspiration by means of an injection-syringe carrying a metal cannula, as used for endometrial aspiration. Compared with the glass pipette which is equipped with a rubber bulb, a more continuous and stronger aspiration is the advantage of this procedure.

4) Obtaining the material by means of a thin wire-loop, the two ends of which are fixed in a bacteriological needle-holder (of the type by Kolle). However, if this kind of collection is chosen, the quantities of material obtained are, in most cases, rather small.

5) The removal of vital cell material from the endocervical walls and glands by cautious scraping with a fine special curet. In this case, predominantly clusters of cells originating from the columnar epithelium will be removed. This material is particularly suitable for cyto-morphological and hormonal studies, the cells showing generally no alterations owing to autolysis or heterolysis.

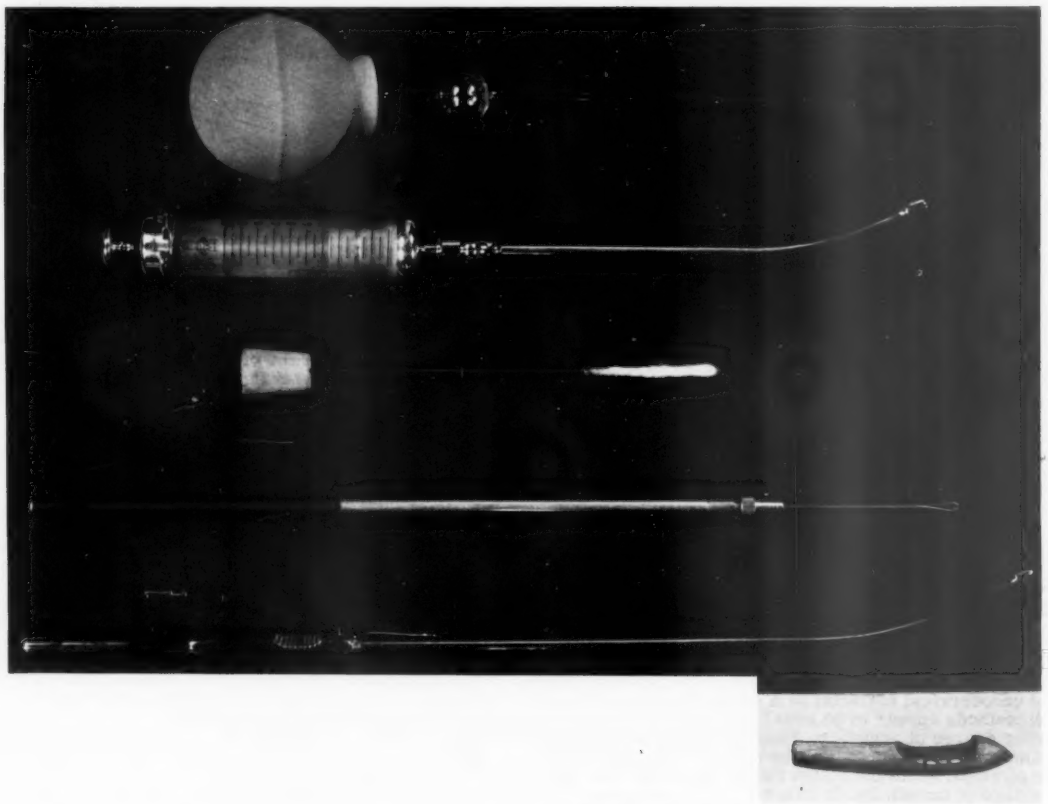


Fig. 1. Instruments for the collection of endocervical cell material.
From top to bottom: Glass pipette with rubber bulb
Syringe with aspiration cannula
Cotton-tipped applicator
Needle-holder with wire-loop for gently scraping the endocervix
Fine special curet
Enlarged tip of the curet (illustrated directly above

3) Collection of material by introducing a cotton-tipped applicator into the endocervical canal and carrying out some rotating as well as upward and downward movements. The stem should be a thin metal stick which, for easier employment, can be equipped with a small bottle-cork serving as a handle. It is important that the cotton-tip should be immersed in physiological saline solution preceding its application; otherwise too much of the cell material can be sucked up and retained by the cotton.

The kind and composition of the smear material depends largely upon the technique of procuring the materials. Though one will aim particularly at obtaining

epithelial cells, in most cases one also has to put up with obtaining considerable quantities of mucus and, in many cases, blood. Often the admixture of erythrocytes can be scarcely avoided, as the columnar epithelium will be easily damaged, following even slight touching with cotton-tipped applicators. Unfortunately, the procurement of abundant vital cell material which has to be loosened from the epithelium is always accompanied by considerable admixtures of erythrocytes. Otherwise the bloodless specimens, having been most gently collected, contain, in most cases, only mucus, devitalized cells and free nuclei owing to plasmolysis. Only if there is a manifest carcinoma of the endocervix, are great numbers of tumor cells easily collected.

There are some additional less important methods of procuring cell material than those mentioned above. Of those mentioned, the use of a cotton-tipped applicator immersed in physiological saline solution seems to be preferable as a routine technique, while, particularly for scientific cell investigations, we have chosen to employ a fine special curet as illustrated.

Bibliography


1. Papanicolaou, G. N.: Atlas of Exfoliative Cytology, Cambridge, Mass., 1954, Harvard University Press.
2. Smolka, H.: Cytomorphologische Untersuchungen am normalen und pathologisch veränderten Cylinderepithel der Cervix uteri, Kiel, 1959, Habil, Schrift.
3. Zinser, H. K.: Die Zytodiagnostik in der Gynäkologie, Jena, 1957, 2. Aufl. VEB Gustav Fischer.


## DISCUSSION

EMMERICH von HAAM, Columbus, Ohio, U.S.A.:

Both authors seem to prefer a moist cotton-tipped applicator as the best means of obtaining an endocervical smear. I would like to ask Smolka about the best way to transfer the material obtained with his fine special curet to the glass slide in order to obtain an even smear. To me the collection of the cervical mucous plug from the external cervical os is not identical with the preparation of an endocervical smear as I gather from Nieburgs' discussion, but rather belongs within the scope of a cervical scraping best performed with an Ayre spatula. To perform a truly endocervical smear the mucous plug of the external cervical os should have already been removed.

LUIS MONTALVO-RUIZ, Madrid, Spain:

We object to the first part of the Smolka paper where he states that the cervical mucus plug can block the pipette, hindering the collection of the endocervical secretions. The canula has the same disadvantage.

For cytological study we reject the use of curettage, which Smolka mentions in paragraph five, and we do not understand why he recommends it for cytomorphological studies, since upon obtaining large sheets of epithelium we cannot see the isolated cells with their typical morphology. Besides, we do not think that it is fit for hormonal readings due to the necessity of taking serial smears, which we do not believe can be done when using the curettage method. For no matter how careful the manipulation, slight epithelial damage will be produced which will lead to a subsequent inflammatory reaction, which is not good for serial hormonal evaluation. For this reason we advise serial vaginal smears.

We agree with Smolka that the best method among all those he mentions is the cotton swab immersed in saline before use, which adequately picks up the cells from the endocervix without trauma. We normally use this procedure.

We agree with Nieburgs that when the cotton swab cannot be put through the cervical canal due to stenosis, one should aspirate with a thin glass pipette or metal canula. By endocervical smears we detect endometrial carcinoma much better than by vaginal or cervical smears and this is the best procedure for accurate diagnosis of endocervical carcinoma.

VIOLETTE M. NUOVO, Paris, France:

We were not as fortunate as Smolka and Nieburgs with the few endocervical smears which were sent to our laboratory.

### TECHNIQUES

Most of them were collected by introduction of a cotton-tipped applicator into the endocervical canal, and sometimes the material was obtained by aspiration with an injection syringe with a metal cannula. In fact, this latter technique was used when the clinician, intending to do an endometrial aspiration, did not succeed in doing so because of a certain stenosis of the endocervical canal.

### RESULTS

Most of the endocervical smears we obtained were covered with large quantities of mucus, in many cases blood, and many free nuclei. They were no help in the diagnosis.

1. Endometrial carcinoma: If the endometrial aspiration is not possible we think it is better to have an endocervical aspiration than nothing. These are the only cases in which we use endocervical aspiration.
2. Endocervical carcinoma: Among our patients there were 45 cases of endocervical carcinoma; 21 were epidermoid carcinoma and cytology was always positive, 24 were glandular carcinoma. We had in this group:
   1) one case where no neoplastic cells were found on the vaginal and cervical smears; maybe an endocervical smear would have been positive.
   2) one case where the vaginal and the cervical smears were negative; the only atypical cells were found on the endometrial smear; they were very few and were probably collected passing through the endocervical canal with the canula.

Apart from these two cases, there were neoplastic cells either on the vaginal smear or on the cervical smear.

### CONCLUSION

Although our experience with endocervical smears is very limited we do not use them because:

1. The results obtained with them were extremely poor.
2. We have good accuracy in endocervical carcinoma diagnosis by using only the vaginal and cervical smears.
3. We cannot screen three or four slides for each routine test; we always ask for a vaginal and a cervical smear, plus an endometrial smear if the patient is postmenopausal.

A. E. RAKOFF, Philadelphia, Pennsylvania, U.S.A.:

Both Smolka and Nieburgs favor the saline moistened cotton-tipped applicator for the endocervical smear.

I personally prefer the dry non-absorbent cotton-tipped applicator for this purpose. When the cervical mucus is copious I often take two endocervical smears, one by aspiration, the other with the applicator. The former smear often contains very few cells but may harbor endometrial cancer cells. I find the endocervical smear far less efficient than the cervical scraping smear for squamous cell carcinoma diagnosis.

H. L. RICHARDSON, Seattle, Washington, U.S.A.:

Dr. Smolka has pointed out 2 significant facts. (1) If the endocervical cells are to be studied, a scraping technique or a fine curet, as he describes, is essential. (2) If a cotton swab is used, it must first be immersed in saline, otherwise the dry cotton soaks up the cells.

Quite in contrast to Smolka, Nieburgs has stated the cotton-tipped applicator probably is the method of choice. Nieburgs advocates the dry smear method and a rehydration technique. I fail to understand what criteria Nieburgs uses for his cytological diagnosis of atypical and/or carcinoma cells. A fixative kills living material and produces a constant artifact. This nuclear artifactual detail allows the cytologist to study the cellular pattern and enables him to render an opinion. I know of no drying technique that does not produce a fusion of the nuclear protein. A good fixative clumps nuclear material in atypical and carcinoma cells. This factor increases accuracy of diagnosis.

In our series of endometrial carcinomas, diagnosed by means of cytology, the importance of the mucous plug as an ideal reservoir for malignant cells cannot be overemphasized. In other sections, I have referred to methods of fixation which clear the mucus and hemolyze the blood.

GEORGE ROMBERG, White Plains, New York, U.S.A.:

Both Smolka and Nieburgs seem to favor the cotton applicator approach for obtaining endocervical smears.

Personally, I favor the aspiration technique using a hard, one ounce rubber bulb and a bevelled glass pipette because with the exception of a few instances where cervical stenosis existed it was possible with this technique to consistently obtain adequate material for cytologic diagnosis.

I would like to compliment both Smolka and Nieburgs on their fine presentations.

## NO CLOSING REMARKS

# REVIEW OF TECHNIQUES OF INTRAUTERINE SMEARS

HERBERT E. NIEBURGS
New York, New York, U.S.A.

It should be noted at the outset that the term "intrauterine smears" may be misleading, since in some cases such smears were attempted with a cotton-tipped applicator with some obvious discouraging results. Possibly, the term: "endometrial aspiration smear" is preferable. The cytologic diagnosis of adenocarcinoma of the endometrium by vaginal and cervical smear techniques has not appeared to be satisfactory. If endometrial cells are found in cervical and vaginal smears, deterioration of cells has occurred and usually does not permit adequate interpretation of nuclear structure. Therefore, under circumstances in which the microscopic evaluation of the endometrial cell changes is mandatory, intrauterine smears should be taken in preference to other methods.

Numerous instruments have been designed for the collection of cellular material from the endometrial cavity. These range from a simple laryngeal cannula and improvised polyethylene tubing, to more complicated devices containing bristles or nylon fibers for abrasion of the endometrial wall. The following instruments are available for use:

1. A metal cannula similar in size to a uterine probe, 6F, 28 cm long, with a Luer-Lock Syringe.
2. A thin malleable laryngeal cannula with beveled edge to which a 5 or 10 cc syringe is attached for suction.
3. A cannula 3/32 inches in diameter, 7 inches long with 4 holes, 1/2 inch in diameter, on two sides of the distal end and a Luer-Lock hub at the proximal end. A stilet with rounded tip is inserted in the cannula, to form a smooth edge at the distal end.
4. A modified insufflation cannula for endometrial aspiration immediately following uterotubal insufflation and hysterography.
5. A small No. 8F pliable polyethylene tube such as is used in the nasal feeding of premature babies. Since it is extremely flexible, it has to be introduced with forceps. A 10 cc syringe is attached for suction.
6. An endometrial polyethylene tube with re tractable bore bristles. Rotation of the bristles in the uterine cavity produces slight abrasion of cells. A selective endometrial specimen without contamination from the cervix may be obtained. This instrument cannot be sterilized by heat.
7. An endometrial cannula with abrasive nylon fibers is composed of a stainless steel handle and a silver malleable cannula. Nylon fibers with slight abrasive properties and suitable for adequate cell adhesion can be extruded, rotated and retracted into the cannula. Before withdrawal, retraction of the nylon fibers into the cannula secures a selective endometrial specimen without contamination by endocervical cells. Sterilization by heat should be avoided.

The direct endometrial smear method affords fresh, well-preserved endometrial cells. Another advantage is that the confusing presence of endocervical cells is eliminated from endometrial smears. In the use of any endometrial cannula, special precautions should be strictly observed. In order to prevent distortion of cells, the cannula should be sterile and dry. Cervical mucous should be wiped away and the cervical canal cleansed with Aqueous Zephiran 1:1000 before insertion of the cannula. If aspiration is used, suction should be released before withdrawal of the cannula in order to avoid contamination with endocervical material.

Evaluation of different techniques for the collection of endometrial cells reveal that the small size Killian antrum cannula for aspiration appeared generally most satisfactory. In comparison with the nylon fiber cannula, the amount of endometrial material collected appeared often more abundant in the smear when aspiration was used, although the nylon fiber abrasive smear resulted in a larger number of satisfactory specimens, particularly in regard to the selective presence of endometrial cells. The reported investigations as to the degree of accuracy with aspiration appears to be about 84% according to Cuyler et al. and Wied. Possibly the use of both aspiration and nylon fiber abrasion may increase the degree of accuracy.

HOWARD L. RICHARDSON
Seattle, Washington, U.S.A.

During the past three years, we have diagnosed 23 cases of carcinoma of the endometrium. In most of these cases, the material was obtained by means of a metal cannula introduced into the endometrial cavity. The method is excellent and 4 cases of adenocarcinoma less

than 10 millimeters in size were in this group. If the method is to be used successfully by physicians in their offices, the use of alcohol and ether is not entirely satisfactory. The drying effect produced on large fragments of endometrial tissue will inhibit staining and prevent later histological sectioning of the tissue fragments. The glycol acetic acid fixative is suitable for both the fixation of smears and biopsy material. The solution clears mucous, penetrates thick cellular clumps and hemolyzes blood. The slides are then stained and the translucency is still preserved. The biopsy fragments in the solution are embedded and sectioned. Wherever possible, a combination of a cytological technique with a histological procedure will increase the accuracy of this diagnostic method. In all cases, every effort should be made to examine all submitted material.

The introduction of a metal cannula into the uterine cavity produces several problems. Adenocarcinoma of the endometrium may infiltrate the myometrium or produce a stenotic obliteration of the endocervical canal. In these cases the cannula may pass through the necrotic tumor into the surrounding tissue spaces. In one case of intermenstrual bleeding, the cannula opened up the vascular channels associated with cystic hyperplasia and hemorrhage resulted. The method is not recommended for those women in the child bearing age group unless pregnancy is ruled out. Since this procedure does require a physician and sterile procedures, there is little likelihood of a serious uncontrolled complication.

GEORGE H. ROMBERG
White Plains, New York, U.S.A.

This paper concerns itself essentially with a description of the endometrial aspiration technique involving a malleable metal cannula, which, because I have found it simpler to use, I prefer to the polyethylene tube and the brush technique.

The equipment used consists of a thin malleable metal cannula with a small tip, which is introduced into the uterus under aseptic conditions after having previously determined the direction and size of the endometrial cavity with a sterile uterine sound; a plain, metal-tip, 10 cc glass aspirating syringe; a one-inch glass tube with a plain metal-tip adapter for blowing out the cannula when the secretions within are too scanty; labeled microscopic slides and a bottle of fixative solution (equal parts of ether and 95% ethyl alcohol).

With the patient in the lithotomy position, the bivalve vaginal speculum usually is sufficient to steady the cervix during the entire procedure. Only in rare instances of obstinate cervical stenosis will it be necessary to apply a cervical tenaculum during the dilatation procedure.

Routinely the endocervical canal is aspirated with a beveled glass pipette attached to a heavy one-ounce rubber bulb. This preliminary removal of cervical mucus aids in eliminating contamination with endocervical cells as does also the use of a Hank dilator in cases where a narrowed canal is encountered.

The metal malleable cannula is easily passed into the endometrial cavity and the tip directed posteriorly to the most dependent portion of the uterus. Gentle aspiration is performed several times. The syringe is then disconnected to release the negative pressure within the cannula. The cannula is then rotated to the left, the syringe reapplied and intermittent gentle pressure exerted as before. Again the syringe is disconnected, the cannula is now rotated to the right and the same procedure is repeated. The aspirating syringe is always disconnected from the cannula when the latter is withdrawn from the uterine cavity, in order to avoid contamination with endocervical elements.

With this technique very little bleeding is encountered and usually no secretions are found in the glass aspirating syringe unless greater pressure has been exerted in order to obtain endometrial fragments for biopsy purposes.

The endometrial secretions in the aspirating cannula are expelled by use of the glass syringe or by the glass mouth adapter when only a few drops of secretion are available. When a dry tap is encountered, the aspiration procedure is immediately repeated.

Eight to ten labeled slides are prepared with paper clips applied to alternate slides to separate them when in solution, and placed side-to-side touching each other as the endometrial secretions are expelled from the metal cannula.

The endometrial smears are always made by blowing secretions on one slide, covering this with a second slide and spreading as done for blood smears. This technique produces thin, even spreads which, when fixed before drying and stained by the Papanicolaou method produces very satisfactory results.

Purulent endocervicitis and acute salpingo-oophoritis are formal contraindications to the performance of intrauterine aspiration. The use of antibiotics and cervical dilatations where indicated, however, expedite the initiation of the endometrial aspiration technique.

Pregnancy also is a contraindication to its use. On a few occasions when the endometrial cavity was inadvertently aspirated in very early pregnancy, I am happy to report that there was no demonstrable injury to the mothers and babies.

The finding of numerous leukocytes and other inflammatory cells in the aspirated intrauterine smears is usually a signal to cease further aspirations and cover the procedure with adequate antibiotic therapy. In the face of a heavy purulent exudate associated with empyema, it is practical to allow for good drainage before obtaining further smears where neoplasm is suspected.

The presence of erythrocytes has not been found bothersome. It has not been necessary to resort to any clearing technique on this account.

The endometrial aspiration technique is a simple, painless procedure which requires a greater degree of meticulousness and experience when used for obtaining intrauterine smears for hormonal evaluation than for cancer screening.

## DISCUSSION

**PIERRE J. HAOUR, Lyon, Rhône, France:**

Dr. Nieburgs gives an excellent review of the different instruments now available for collecting endometrial cells. Romberg gives useful details on the technique of intrauterine aspiration.

I want only to mention two important variances to the techniques which can be used successfully in some circumstances.

When the exudate obtained is too scant we used the technique of endometrial washing proposed by Morton. When the exudate, on the other hand, is heavy, as in inflammatory reactions, or in cases of hemorrhagic material, the exudate or the blood after aspiration, is mixed with saline and centrifugated. In our hands these methods have given excellent results.

The advantages and disadvantages of intrauterine techniques have already been discussed in a former issue of Acta Cytologica. Until now it seems difficult to evaluate, in the diagnosis of adenocarcinoma, the difference in accuracy obtained with one instrument or another, or with their association (cannula and intrauterine brush). If the intrauterine brush obtains, as was stated, more preserved material, it might give better results than the ordinary cannula.

Bibliography

1. Morton, D. C.: Eleventh International Congress of the International College of Surgeons, Transaction, 1959.

ABRAHAM E. RAKOFF, Philadelphia, Pennsylvania, U.S.A.

Dr. Nieburgs has listed at least 7 different techniques for endometrial aspiration. In our experience it is well to have several of these available for choice, depending on the local findings in the particular patient. In addition, probes and small Hegar type dilators should be available for the many instances in which the cervix is stenotic and the internal os must be opened before even the smallest of the endometrial aspiration instruments can be used.

Dr. Richardson has pointed out the dangers of the intrauterine smear. These clearly indicate why this technique is not recommended for "routine" use. On the other hand, the dangers should not be greater than those attendant upon "sounding" of the uterus and the precautions to be followed are the same. Whenever the history suggests the possibility of fundal cancer, endometrial aspiration is worthwhile since it uncovers some cases which are missed on the other smears. In our laboratory it was found that the correct endometrial suspicious cases were improved from 66 to 92% by adding endometrial aspiration to the vaginal and cervical smears in properly selected cases.

## NO CLOSING REMARKS

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

# MATERIAL OBTAINED BY PIPETTE FROM VAGINAL POOL ONLY, WITHOUT VISUALIZATION OF UTERINE CERVIX

EMMERICH von HAAM AND
EDWARD M. MILLER
Columbus, Ohio, U.S.A.

Obtaining material by vaginal aspiration is the method of choice in large survey projects of certain geographical areas for the detection of unsuspected carcinoma of the cervix among the general population. For the past 3 years we have been conducting such a survey project under the auspices of the National Cancer Institute and have to date examined 90,639 women. Many patients were examined by private physicians who prepared vaginal smears after visualization of the cervix by speculum. All clinic patients, however, and patients of state institutions and from industrial concerns were examined only by the vaginal aspiration technique without visualization of the cervix. For this purpose girls were trained as smear takers, and usually two vaginal smears were examined from each patient.

The advantages of this method are chiefly in its simplicity. We are always able to find persons willing to be trained in the technique and to fulfill this task at a comparatively low level of remuneration. Thus by this method the expensive and limited time of a physician can be spared, since the smear takers are also able to take the relatively simple historical data required from the patients. The smears are then screened by trained cytotechnologists, checked by experienced cytotechnologists, and the only time a physician is involved in the Cancer Survey Project is when the pathologist examines the doubtful and positive smears submitted by the checking cytotechnologist and refers those cases to the gynecologist for biopsy and eventual treatment. Even in private practice many physicians train their office nurses in the technique of obtaining vaginal aspirations without the use of the speculum and thus are able to handle a larger number of patients. Another advantage is the time factor involved in the procedure. The taking of the history of the patient and obtaining the smear by the vaginal aspiration method require about 5 minutes of the patient's time. This factor becomes valuable publicity material in inducing women to visit their doctors or the various clinics set up for this purpose at the various hospitals. It also convinces many managers of industrial concerns that their employees will lose little time and influences them to give permission for their women to visit the industrial nurse during working hours in order to have this examination. In fact, an educational movie entitled, "Three Times Five Minutes," stressing the short time involved in this procedure, is used advantageously by the local unit of the American Cancer Society to publicise the cancer survey.

Against these three advantages—simplicity, economy, and the time factor—are the two disadvantages frequently noted by various writers (1). The one of possible injury to the vagina by this method was found to be negligible in our experience. Only once did a woman claim to have been "hurt" by the introduction of the pipette by a smear taker, and once a pipette broke in the vagina and had to be extracted by a physician, without injury to the patient. The other disadvantage is that of greater inaccuracy inherent in this method. In order to investigate this more thoroughly, we tabulated the false negative examinations obtained in our survey thus far (Table I). Of 398 tumors encountered in the past 3 years, 66 tumors were missed because the women were examined only by the vaginal aspiration method. Only 19 tumors were missed in women examined by cervical smear method with the use of the speculum. Therefore we obtained three times as many false negative results by using the "blind" vaginal aspiration method. The greatest difference between the two methods was in the group of 152 invasive carcinomas, in which 21% were missed by the vaginal smear method as compared to 5.3% by the cervical smear method. Had we examined all women by the cervical smear method we would have had an over-all accuracy of 95.2% as compared to an over-all accuracy of only 79% in our present survey. This difference is quite serious and calls for an explanation.

We feel that there are two factors which contribute to the larger number of false negatives obtained by the vaginal aspiration method without the use of a speculum. One is the fact that many patients, although explicitly instructed to the contrary, will take a douche before they report to their physician or to the clinic for examination. This will remove all accumulated cells, and too few cells will exfoliate into the vaginal pool during the relatively short interval. The second source of errors can be found in the smear takers. Since this procedure is a blind one, and the smear takers are relatively inexperienced in intravaginal manipulations, it is very likely that many times the pipette is not placed into the proper position to aspirate the vaginal pool. Usually at the first slight resistance produced by the vaginal folds or a protruding cervix, the smear taker will stop and aspirate the secretion of the vagina rather than of the vaginal pool. Since only very little material is needed to prepare the slide aspiration, any area of the vagina will often suffice, and thus the important spot from which a correct vaginal aspiration should be taken is missed.

Our Table also shows the interesting fact that

Table I

| Type of Tumor | Total Number Malig. Tumors | Diag. missed by Vag. Pool Aspirate | | Diag. missed by Cervical Smear | |
|---|---|---|---|---|---|
| | | No. | % | No. | % |
| Carcinoma in situ | 186 | 18 | 9.7 | 4 | 2.2 |
| Invasive carcinoma | 152 | 32 | 21.1 | 8 | 5.3 |
| Endometrial carcinoma | 47 | 13 | 27.7 | 7 | 14.9 |
| Other malignant tumors | 13 | 3 | 23.1 | 0 | 0 |
| | 398 | 66 | 16.6 | 19 | 4.8 |
| | | Total | Average | Total | Average |

more invasive carcinomas have been missed than carcinomas in situ. This can be explained by the fact that the surface of an invasive carcinoma is often covered with necrotic detritus and that malignant cells are not as easily obtainable (1). The lesser accuracy of this method in detecting endometrial carcinoma is well known and needs no further comment.

In weighing critically the advantages and disadvantages in this method, we still feel that vaginal aspiration should be recommended in those instances where mass screening of a certain population is to be accomplished, since even an 80% accuracy will detect enough cancers to make this method worth while. On the other hand, we do not recommend this method to the private physician to be used as his office procedure. As he must spend considerable time with his patient anyway, he should use the speculum and take smears from the cervix as well as from the correctly visualized vaginal pool in order to obtain better results. In a well organized office such a procedure will take approximately 15 minutes, and we feel that the additional 10 minutes of the physician's time is wisely spent for a more accurate examination.

Bibliography

1. Smolka, Hans and Soost, H. J.: Grundriss und Atlas der Gynakologischen Cytodiagnostik. Stuttgart, 1956, Georg Thieme Verlag.

## DISCUSSION

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany:

As von Haam and Miller mentioned, we were already previously against the "blind" taking of smears, because the method shows unreliable results in detecting cancer (1). The examination of the authors proves this distinctly. We also stated that "blind" aspiration failed even in cases of clinically recognizable carcinoma.

The removal of the pipette from the fornix of the vagina without viewing the ectocervix gave, in the examinations of von Haam and Miller, false negative findings in 16 (6%) of the cases. In more than two-thirds of these cases the carcinoma would have been discovered by viewing the cervix and direct taking of the smear!

Even in connection with preventive examinations we ask ourselves whether or not "blind" aspiration can be justly recommended. In our opinion it is not! One is not allowed to forget how dangerous the illusive safety is in which the women are lulled by a flase negative cytological finding. Many of them would delay coming to the doctor upon noticing the first symptoms.

Only if it is impossible to visualize the ectocervix or if women refuse a gynecological examination is it right, in my opinion, to take a specimen by "blind" aspiration or by tampon.

The cytological examination is by no means a "test" which can be performed like a chemical reaction in laboratory. As a tracing method the cytodiagnosis can only be a guide to an improvement of our results in the early diagnosis of genital cancer, if it is used as a complementary method and if it is accommodated to the methods of diagnosis already practiced.

Bibliography

1. Smolka, H. and J.-J. Soost: Grundriss und Atlas der gynäkologischen Cytodiagnostik. Stuttgart, 1956, Georg Thieme Verlag.

MARCEL GAUDEFROY, Lille, Nord, France:

Advantages and inconveniences of the use of "blind" vaginal aspiration by pipette have been perfectly explained by von Haam and Miller. I agree with them entirely. The inconveniences seem so important to me that I never use that technique in married women. Only in girls, or virgin women, do I use the pipette without speculum. In hormonal evaluation, I often find false eosinophilia.

J. PAUL PUNDEL, Luxembourg, Luxembourg:

I have to congratulate von Haam and Miller for their logical and exhaustive study of this problem, and I agree completely with their conclusions. Blind aspiration should be strictly reserved for mass screening by nurses or technicians, but there is no place for it in the doctor's office. If every general practitioner, called to treat gynecological cases, would take the time to use a vaginal speculum and look at the cervix and send all abnormal cases to a gynecologist for further examination, I think we would have won an important battle against cancer. In addition to the speculum examination, the collection of a cytological smear takes so little time, that this small disadvantage is counterbalanced by the advantages the doctor can offer to his patients concerning their health. However, this screening in the doctors' office needs perfect organization with central laboratories.

## CLOSING REMARKS

EMMERICH von HAAM:

I agree with Bickenbach and Soost in their strong stand against the "blind" aspiration method. They pointed correctly to the great danger inherent in this method by lulling women into a false and illusive feeling of safety. I am glad that so experienced men as Gaudefroy and Pundel are in agreement with our conclusions.

# MATERIAL OBTAINED BY PIPETTE FROM VAGINAL POOL ONLY, AFTER INSERTION OF SPECULUM

LUIS MONTALVO-RUIZ
Madrid, Spain

Our initiation into the study of vaginal cytology began in 1941 after reading the work done by Botella and Asin (3). Following their technique, we obtained material with a platinum loop from the middle portion of the vagina after introduction of a speculum. Later, in 1942, after reading the work by Mack (5,6,7), we obtained the exudate with a cotton swab, and later when we read the monograph by Papanicolaou and Traut (9) we began to use the aspiration pipette technique. Later we began to use the Ayre spatula (2) which we use today, for ruling out carcinoma of the uterine cervix.

Now for hormonal diagnosis we exclusively employ the use of a pipette, following introduction of a bivalve speculum in order to visualize the vagina, with the exception of young girls, virgins or those cases where the introitus is stenosed, whether by radiation or other pathological processes. In these cases (with the exception of young girls), we also utilize the pipette preceded by a virginal retractor which improves the introduction of it.

For obtaining material we follow the classical technique as indicated by Papanicolaou (9), Allende (1), the Vincent Memorial Hospital (12), etc. Following the introduction of a speculum, we directly aspirate from the lateral cul du sac with a completely dry pipette and gently sweep the vaginal wall towards the periphery in approximately a three centimeter extension. We then loosen the bulb, being very careful not to aspirate from the external third of the vagina. The material is projected onto the slide using the same pipette with repeated squeezing of the rubber bulb. With this method the relationship that the desquamated cells bear to each other is minimally altered. We believe that this method is ideal for the study of hormonal cytology. The preceding conclusions were arrived at by the University Cancer Prevention Clinic of Professor Botella-Llusia. Three specimens were obtained from the same patient; one from the vagina with a pipette, another from the cervix with a spatula, and another from the endocervix with a cotton swab. We have observed that the cells were better preserved when using the pipette and at the same time better spread than those obtained with a spatula. In view of this observation, we have given to an intern of the Clinic the task of obtaining samples in 100 patients using the spatula and the pipette (in the same patient) for preparing smears from the right and left cul du sacs. From this study the following results were observed: in 55% of the cases, the morphology of the cells was much better presented when obtained with a pipette than with a spatula. With the latter, leukocytes, red blood cells and exfoliated cells appeared stretched and folded, displaying pseudo-folds that were not seen when the pipette was used. This can be seen in Figures 1 and 2 from the same patient.

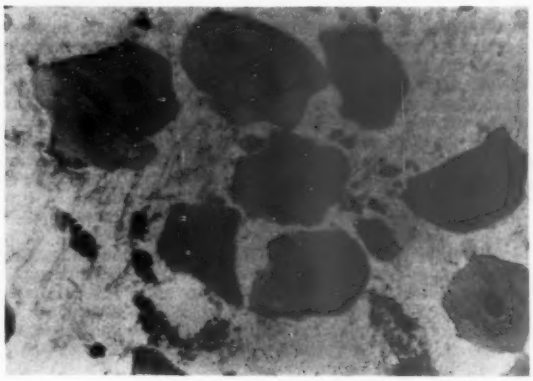


Fig. 1. Taken with a pipette.

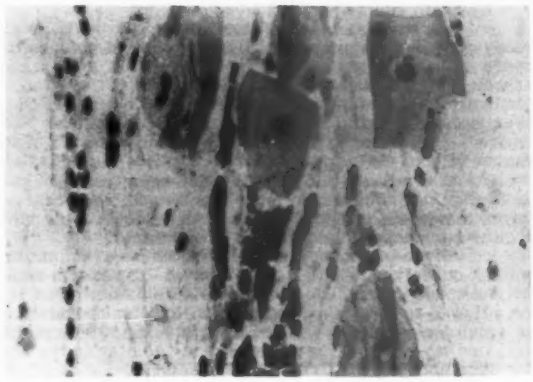


Fig. 2. Taken with a spatula on the same patient.

Thus, we believe that for the study of hormonal cytology the method of choice is to obtain the material with a pipette from the lateral cul du sac, since this is a method that modifies the cellular morphology less, a fundamental factor in hormonal diagnosis. For the study of malignant cytology, we obtain the material from the posterior cul du sac with a pipette or, better, with the spatula. Obtaining material from the posterior cul du sac for hormonal diagnosis has the inconvenience of stasis, which produces necrosis of exfoliated cells and debris, with the accumulation of cervical mucus and isolated cells of ectocervical origin that may give rise to a false hormonal interpretation (10,11). We discarded the platinum loop recommended by Rauscher and the "sponge biopsy" introduced by Gladstone (4).

Bibliography


1. deAllende, I. L. C. and Orias, O.: La Citrologia vaginal humana. Buenos Aires, 1947, Hoeber.
2. Ayre, J. E.: Cancer Cytology of the Uterus, New York, 1951, Grune Stratton.
3. Botella, J. and Asin, J.: Medicine Espanola, Aug. 1941.
4. Gladstone, S. A.: Amer. Med. J. 5:849, 1948.
5. Mack, H. C.: Harper Hosp. Bull. 1:54, 1942.
6. Mack, H. C.: J. of Clin. Endocrinology, Mar. 1943.
7. Mack, H. C.: Amer. J. of Obst. & Gyn. 45:402, 1943.
8. Montalvo, L. and Sloker, C.: Acta Gin. 2:183, 1951.
9. Papanicolaou, G. N. and Traut, H.: Diagnosis of Uterine Cancer by the Vaginal Smear. New York, 1943, Commonwealth Fund.
10. Pundel, J. P.: Acquisitions recentes en Cytologie Vaginal hormonal, 1957.
11. Rauscher, H.: Ginecologia (Mundi), Buenos Aires, 1956.
12. Vincent Memorial Hospital: The Cytologic Diagnosis of Cancer, Philadelphia, 1958, Saunders.


## DISCUSSION

MARCEL GAUDEFROY, Lille, Nord, France:

I agree with Montalvo Ruiz that the pipette in non-experienced hands is theoretically the most adequate to avoid false folding of cells, but the pipette aspiration often gives large masses of badly colored, useless cells. After introduction of a bivalve speculum, my experience has shown that it was preferable to use a metallic or wooden spatula in order to obtain a very well spread smear, and a very experienced, very nimble hand does not produce pseudo-folded cells, if the spreading of the smear is light and quick. I reserve the pipette aspiration for occasions when the use of a speculum is impossible.

J. PAUL PUNDEL, Luxembourg, Luxembourg:

The aspiration technique gives excellent results if the vaginal content is abundant, but, as I have mentioned in this Symposium, if the vaginal content is scanty, it is often very difficult to obtain sufficient material by pipette aspiration. Personally, I prefer a wooden tongue blade for the collection of vaginal smears. I have not found evident changes in the vaginal cells as a result of this collection technique, but I think that this is only a question of technique. If one uses completely dry wooden spatulas, cell alterations may occur by the strong movements necessary to separate the cells from the blade, where they adhere very strongly due to the adsorption of the liquid into the wood. I have eliminated this difficulty by moistening the blades shortly before taking the smear, and the collection is done by passing the blade in a very gentle manner over the vaginal epithelium. For the rest, I agree completely with Montalvo Ruiz.

## CLOSING REMARKS

LUIS MONTALVO-RUIZ:

I have made my observations on material taken with a wooden spatula. Some errors probably can be avoided by moistening the wooden spatula as Pundel has suggested. Henceforth I shall try out this technique which is very interesting to me. The preparation of smears with a wooden spatula has advantages over the aspiration with a pipette because it is faster. However, it may be a question of habit. Sometimes, when the vaginal fluid is thick the aspiration technique picks up sheets of crowded cells which stain very poorly. In order to avoid this, we dilute the fluid with a drop of saline over the slide, blowing several times with the rubber bulb until a uniform and thin smear can be obtained before fixation and staining.

# MATERIAL OBTAINED BY VAGINAL TAMPON ONLY

WERNER BICKENBACH and
HANS-JÜRGEN SOOST
Munich, Germany

HERBERT E. NIEBURGS
New York, New York, U.S.A.

In our experience the detection tampon technique is less efficient than the direct smear technique. The reasons are as follows:

1. In many instances, advanced carcinomas do not shed atypical cells when the surface of the ulcer crater is extensively necrotic. On direct examination with a speculum these cases are readily recognized and thus the value of cytodiagnosis is not questioned to any extent. Tampon technique alone may, however, not detect the carcinoma and the negative finding will erroneously put the patient at ease.

2. The tampon technique gives negative findings more frequently than the direct cervical smear. This is particularly noticeable when the patient herself introduces the tampon; the tampon may not be inserted deeply enough and thus does not arrive at the region of the cervix.

In 65 of our cases the tampon technique gave 13 cases in which there was a negative finding, while the smears, taken directly from the cervix, showed atypical cells in all of these cases, or a carcinoma was already clinically recognizable.

We, therefore, are of the opinion that the tampon technique should be employed only when the patient refuses gynecological examination or if this is not possible for one reason or another. It is obviously better to make use of the tampon technique in such cases, rather than to do without cytodiagnosis at all. Since the results are poorer when the patient herself introduces the tampon, it seems advisable that this be done by someone medically trained.

### Bibliography

1. Soost, H. J. and Nevin, R.: Medizinische, in press.

In an effort to extend detection of carcinoma of the uterine cervix to a larger population group, several tampon methods have been developed. The tampon designed by Draghi, and tested by Brunschwig, Papanicolaou, Thornton and others, consists of a cylindrical core of tightly compressed cotton encased in a sheath of nylon. The latter is non-absorbent and when inserted into the vagina permits retention of material on the surface for a smear. When the tampon is removed from the vagina, the material can be smeared upon glass slides, and the slides are then inserted into a fixative before staining. Out-patients coming into the office were afforded the necessary privacy to insert the Draghi detection tampon themselves. After a period of from 5 to 30 minutes, in the waiting room, the tampons were removed during the course of physical examination and smears made on clean glass slides. The smears were made by stamping the distal end of the tampon against the slide, with or without slight clockwise rotation.

The accuracy of the Brunschwig-Draghi tampon appears to be 80%. Out of 65 patients with carcinoma, 52 were positive. However, there was a group in which a diagnosis of "deferred classification" was made, and if this group is counted as positive, then 63 of 65 patients, or 97%, were accurate. In addition, there were 18 of 130, that is, 13.8%, without cancer who were reported as "deferred classification." Thornton obtained a correlation of biopsy and cytology in 90.5%.

In our studies, certain disadvantages inherent in the tampon method were observed when women were requested to prepare their own smears. It was found that the tampon, when inserted, was situated in various positions in relation to the cervical os. While the instructions required preparation of the slide from the tip of the tampon, the cervical material was often situated at the side of the tampon.

In an endeavor to obtain satisfactory cervical material regardless of the site of location on the tampon, a device was constructed for simplification of the technique. With this method, the tampon after removal from the vagina was pulled through a container between two glass slides. In this manner, material was obtained from a large surface area of the tampon. In two separate studies it appeared that the co-operation of women for the use of this device could be obtained in only 1/3 of the cases. Of the women who used the tampon, 1/3 submitted unsatisfactory specimens. On the whole, the microscopic evaluation is time consuming due to the increased crowd-

ing of material from the cervix and vaginal pool and/or the presence of many deteriorated cells or the larger area of examination in our modification.

In order to further improve this method of cell collection, additional modifications have been made, designed to reduce the amount of instructions to the women to a minimum and to avoid deteriorated cells in the smear due to prolonged collection. This modified tampon applicator which was recently tested consists of a cardboard tube which is inserted into the vagina and which contains in its lumen a cone-shaped plastic slide. The smear was collected from the cervical area by means of an inner plunger to which a nylon fiber tampon is fitted. In the process of removing the inner plunger, a smear imprint is made on the plastic slide. The plastic slide is stained in a regular manner and then mounted between two glass slides for microscopic examination.

This device was tested on 2,500 women and revealed a larger number of satisfactory specimens than obtained with the ordinary tampon method; however, the microscopic examination of the slides remained a time-consuming procedure. This difficulty has recently also been eliminated by a further modification and is now undergoing investigation, the results of which will be reported at a later date.

In conclusion, it appears that none of the tampon methods described and tested up to date have proved to be satisfactory for the purpose for which they are designed.

## DISCUSSION

GENEVIEVE BADER and EMERSON DAY, New York, New York, U. S. A.:

The tampon smear must be judged in the light of the purpose for which it is intended to be used. If it is designed to be used in mass screening of a population it serves its purpose. In a study of 2,694 patients at the Strang Clinic it was found that the tampon smear was about 90% accurate (1). The tampon smear was shown to be more accurate than a single vaginal smear but less accurate than the combined vagino-cervical smear obtained by the direct smear technique. The advantages of the tampon smear are chiefly those of ease, saving of time, minimum use of physician time and appeal to the patient. In any large scale screening program it would be preferable to use the tampon technique rather than to rely on a single vaginal smear. For routine smears in a clinic or in the office of the private physician the direct smear technique is obviously the best.

In the previous discussions mention is made that the tampon smear technique may miss cases of advanced carcinoma. This is true and represents one of the inherent errors of this method of cell collection.

### Bibliography

1. Bader, G. M., Simon, T. R., Koss, L. G., and Day, E.: Cancer 10:332, 1957.

## CLOSING REMARKS

WERNER BICKENBACH and HANS-JÜRGEN SOOST:

We agree with Genevieve Bader and Day, that the tampon method for mass population screening may be an additional aid. One should not forget, however, that the tampon method is only an expedient by which the diagnostic accuracy of the direct smear in combination with the clinical examination cannot be reached.

Concerning adenocarcinoma of the corpus uteri, we believe that there may be a certain superiority of the tampon method, agreeing with Brunschwig and Papanicolaou, as well as with Scott, Brown and Reagan. One may explain this by the fact that the corporal cells will be passed through the cervical canal only rhythmically and are not continuously exfoliated as is the case with cells from a cervical carcinoma. The chance of finding corporal cells in a tampon which lies for several hours close to the ectocervix could be better than by taking one direct smear from the ectocervix.

### Bibliography

1. Brunschwig, A. and Papanicolaou, G. N.: Cancer 10: 120, 1957.

2. Scott, R. B., Brown, A. M. and Reagan, J. W.: Am. J. Obst. & Gyn. 73:349, 1957.

# MATERIAL OBTAINED BY CERVICAL SCRAPING ONLY

FRIEDRICH BAJARDI
Graz, Austria

There is no doubt that limiting cytological collection to only one method will result in a decrease in accuracy. The results will be inferior, especially in endometrial and tubal carcinomas, particulary if one renounces the aspiration methods.

If one searches primarily for cervical carcinomas, the cervical scraping method is very accurate (6). The absence of other methods will not weigh too heavily in this case.

At the University Women's Hospital at Graz we strive primarily for the early detection of cervical carcinomas. Thus, every patient is submitted to a colposcopical examination and a cytological smear, prepared by the scraping method.

The advantages and disadvantages of limiting cytology to this one method of obtaining specimens may be discussed regarding our own material. The results of the scraping method are submitted to a certain control by the simultaneous application of colposcopy. The final results are based upon histological verification. Biopsies are done in each case with colposcopic suspicion as well as with positive or suspicious cytology. Moreover, the accuracy of colposcopy and cytology is controlled to a certain degree by histological examination of every single surgical specimen. If, for instance, in a case of uterine myomas, colposcopical and cytological findings have been reported negative, the cervix of the removed uterus is still examined histologically.

Surface carcinomas and early invasive carcinomas, which we classify in one group as "preclinical carcinoma" (4), have been detected with the (controlled) scraping method in about 90% of the cases. This result has been constant over a period of several years (1,2,3, 5). If we do not consider the error of the examiner but the value of the scraping method as to how often the smear, and not the examiner, signals a carcinoma, we arrive at an accuracy of about 95%.

Besides this remarkably high accuracy in picking up "preclinical carcinomas," we would like to emphasize some other advantages of this method:

1. Simplicity and inexpensiveness of the equipment.
2. Necessity of visualizing the cervix. Thereby, the eye becomes trained in recognizing suspicious cervical lesions.
3. Cleaner and therefore easier to screen smears than, for example, aspirated material. Also atypical epithelia are more abundant in scrapings than in aspiration smears.
4. Possibility, under guidance, of taking material from suspicious sites of the uterine cervix.
5. Cytodiagnosis is not limited to an examination of the desquamated cells from the surface alone, but also brings cells from the deeper layers of a squamous epithelium. This may be of importance for the appreciation of a basal cell hyperplasia.

As an essential disadvantage of the scraping method, we consider the low accuracy in detection of corporal carcinoma, which in our material is only about 30%.

Bibliography

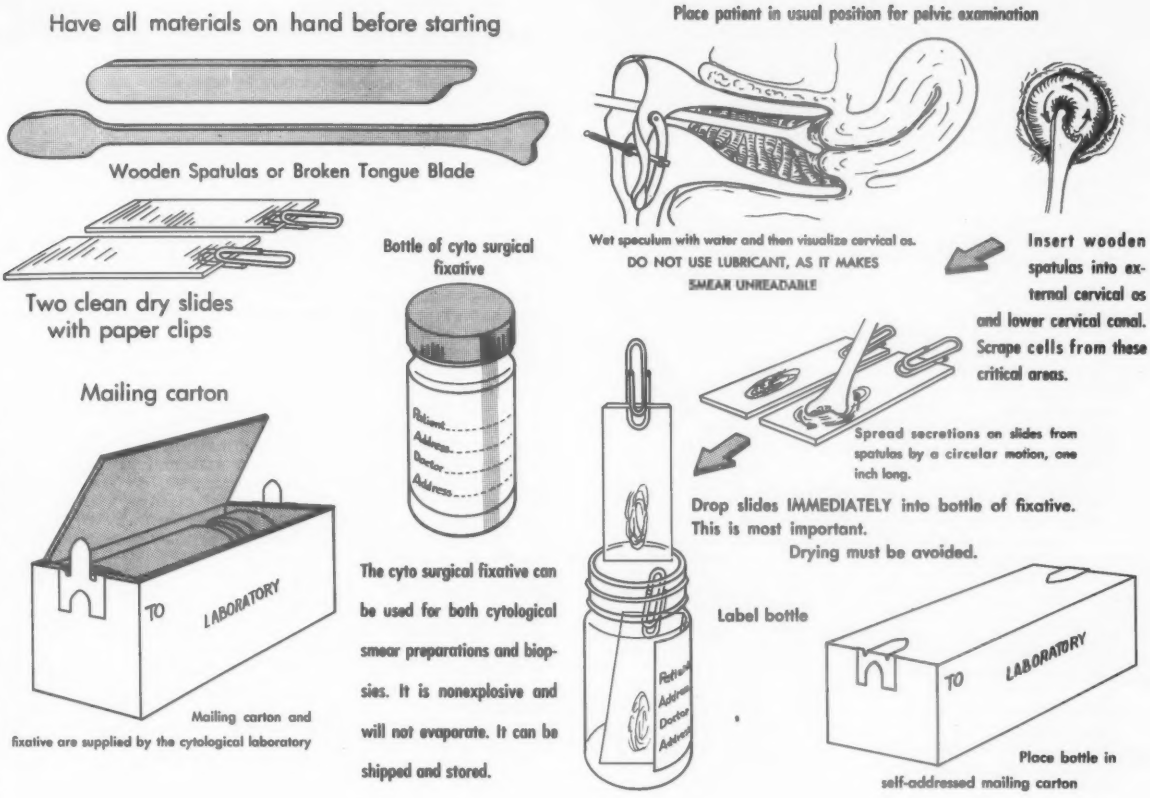
1. Bajardi, F., Burghardt, E., Kern, H. and Kroemer, H.: Gynecologie Pratique, to be published.
2. Burghardt, E.: Wien. Klin. Wschr. 69:253, 1957.
3. Burghardt, E. and Bajardi, F.: Arch. Gynak. 187:621, 1956.
4. Navratil, E.: Biologie und Pathologie des Weibes, Seitz-Amreich, vol. 4, Berlin, 1954, Urban & Schwarzenberg.
5. Navratil, E.: Acta Union Internat. contre le Cancer 14:346, 1958.
6. Wied, G. L.: Am. J. Clin. Path. 25:742, 1955.

HOWARD L. RICHARDSON
Seattle, Washington, U.S.A.

The scraping of the cervix with a wooden tongue blade is the most satisfactory technique applicable to physicians' offices. A recommended method is illustrated in the series of diagrams shown on the following page.

The fixative used by us is non-combustible and can be shipped through the United States mails. It has the following formula: (For approximately 10,000 cc of fixative)

3446 cc monoethylene glycol
181 cc diethylene glycol
36 gm borax pentahydrate
5776 cc water
500 cc acetic acid


Have all materials on hand before starting
Wooden Spatulas or Broken Tongue Blade
Two clean dry slides with paper clips
Mailing carton
TO LABORATORY
Mailing carton and fixative are supplied by the cytological laboratory
Bottle of cyto surgical fixative
Patient
Address
Doctor
Address
The cyto surgical fixative can be used for both cytological smear preparations and biopsies. It is nonexplosive and will not evaporate. It can be shipped and stored.
Place patient in usual position for pelvic examination
Wet speculum with water and then visualize cervical os.
DO NOT USE LUBRICANT, AS IT MAKES SMEAR UNREADABLE
Insert wooden spatulas into external cervical os and lower cervical canal. Scrape cells from these critical areas.
Spread secretions on slides from spatulas by a circular motion, one inch long.
Drop slides IMMEDIATELY into bottle of fixative. This is most important.
Drying must be avoided.
Label bottle
TO LABORATORY
Place bottle in self-addressed mailing carton

The following table is an analysis of the results obtained by the scraping method used in physicians' offices.

Advantages of this method over all others are briefly listed. It is part of the routine physical examination. It requires no special preparation. The atypical or positive cytology can be repeated. The abundance of cell flora shortens the screening time. Positive diagnosis in the first smears has proven to be of practical value in patient therapy and shortens the time between smears and confirmatory biopsy for cancer.

In our experience, there were 11 false negative cases in 19,662, or 0.6 cases per 1000.

## DISCUSSION

CLARICE DO AMARAL FERREIRA, Rio de Janeiro, Brazil:

I agree with the main speakers that we miss more cancer cases with the vaginal smear only than with the cervical scraping. In the "Instituto de Ginecologia" (Rio de Janeiro), the screening is usually done on the material obtained by cervical scraping, the results controlled by colposcopy and histology. Only in rare cases, as in virgins or a stenosed vagina, do we take vaginal smears

Table I

| | 1956 Genital Cases 3045 | | 1957 Genital Cases 5974 | | 1958 Genital Cases 10643 | |
|---|---|---|---|---|---|---|
| | | per 1000 | | per 1000 | | per 1000 |
| Abnormal Cases | 105 | 34.1 | 148 | 24.8 | 219 | 20.6 |
| Total proven positive cases | 47 | 15.4 | 55 | 9.2 | 72 | 6.8 |
| Total positive in first smear | 33 | 10.8 | 37 | 6.0 | 54 | 5.0 |
| Total false negative | 1 | 0.3 | 6 | 1.0 | 4 | 0.4 |

only. We have never used the tampon smear as a screening method. We think the cervical scraping method is the best. I believe it has the same disadvantage for detection of corporal cancer as does the vaginal sample; in suspicious cases we do an aspiration smear of the uterine cavity, which diminishes the cause of errors. This would have to be a routine examination also for detection of cancer in situ of fundus.

PIERRE J. HAOUR, Lyon, Rhône, France:

It is obvious that the best technique for collecting cancer cells is to take them as near as possible to the site of desquamation. Concerning cancer of the cervix, the scraping method is the best procedure as is well shown by the results of Bajardi and Richardson and also by the work of others.

In their paper the authors did not indicate which type of material is obtained by this procedure. In fact, one can find in smears made in the vaginal pools all the cells encountered on the cervix; the cervical smears, however, exhibit a rather distinct pattern. The normal cervical smears obtained by scraping consist mostly of transitional cells and of a smaller number of intermediate and superficial squamous cells of the vaginal type (but with a lower Karyopyknotic Index). Columnar cells from the squamo-columnar junction are present, but increased in number in ectopy and are mixed with transitional cells.

In cervical lesions the smears can be done by scraping limited to the lesion, under visualization by means of the speculum or of the colposcope. Lugol's solution or acetic acid applied previously on the cervical mucosa does not produce any significant cytological alterations. Anucleate squames are often found in Lugol-negative zones, prolapse and leukoplakias.

Endometrial cells are not often encountered in normal women, even after applying a cervical pessary (1). The accuracy of cervical scrapings in the detection of endometrial carcinoma varies with the authors, better results being obtained with cervical aspiration at the external os of the cervix; but this procedure has a lower accuracy than endometrial aspiration and even than vaginal smears (30% according to Bajardi's experience).

In our experience a combination of both scraping and aspiration in adenocarcinoma has given us an accuracy of 73%.

As it is shown in other papers of this issue, cervical scraping gives the best result in cancer of the cervix.

Bibliography

1. Bajardi, F.: ACTA CYTOLOGICA 2:577, 1958.

WARREN C. HUNTER, Portland, Oregon, U. S. A.:

To Richardson:

While the accuracy of cancer detection appears to be gratifyingly high and the false negative results very low, which speaks well for the cervical scraping method of obtaining material, the reviewer's experience is contrary to the statement that "the abundance of cell flora shortens the screening time."

The great likelihood of dilution of squamous cells by quantities of mucus, the presence of so many endocervical epithelial cells, singly and in groups, and the frequency with which erythrocytes are a component of these smears tend to scatter the squamous epithelium and to prolong the search instead of shortening the study time.

The other advantages mentioned by the author are found in specimens taken from the posterior fornix and are not peculiar to cervical scrapings.

My personal preference is still for two smears, one from the posterior fornix, the other from the cervical os.

To Bajardi:

It is agreed that limiting cytological collection to a single method will decrease the accuracy of finding cancer cells. It is also agreed that colposcopical examination of the cervix by a trained observer should add valuable information in the search for abnormal areas. Certainly the author is to be commended for controlling smear results with histological examination of cervical tissue. The 90-95% accuracy of the method speaks for itself as a means of detecting cervical cancer.

The reviewer is not in agreement with Bajardi that cervical smears obtained by scraping are cleaner and easier to screen than aspirated material and that a cervical smear alone will not weigh too heavily against finding cancer in this location. My personal preference is for two smears in each instance, one from the posterior fornix, the other from the cervical os. In my experience the frequent masses of endocervical epithelium, together with abundant mucus and often many erythrocytes serve to dilute the squamous epithelium and require a longer search than is needed for material taken from the posterior fornix. Personally, I would rather not attempt to distinguish between normal basal cells in groups and hyperplastic basal cells as suggested by Bajardi. In general, cells in groups are not well suited for accurate study except at the periphery.

ALEXANDER MEISELS, México, D. F., México:

The high accuracy and the advantages of this method, as stated by Bajardi, have made its use very wide-spread. If a large detection program should include cytologic screening by only a single smear, the cervical scraping would be very much indicated. Nevertheless, one should hesitate to limit the scope of exfoliative cytology by using only one single method. Even if this method proves to be the most accurate in detecting cervical carcinomas, it will miss a considerable number of endometrial adenocarcinomas, and the information it gives as to hormonal levels, concomitant infection or trichomonad infestation, etc., is not as reliable as that provided by the various vaginal smears. One of the great advantages of exfoliative cytology (when vaginal smears are included in the study) is that cells from almost all of the genital tract are brought under observation. This provides for a very valuable over-all picture, and often points out the direction in which further studies should be done.

Cervical scraping alone offers relatively few advantages over a well done ring biopsy of the cervix, and represents a serious limitation to the scope of exfoliative cytology.

LUIS MONTALVO-RUIZ, Madrid, Spain:

In another paper in the present Symposium, we have studied the advantages and disadvantages of the cervical smear. Our conclusions agree with the positive findings of Bajardi, but we must insist that the cervical smear alone is insufficient for the hormonal diagnosis because the presence of endocervical mucus may give rise to pseudoeosinophilia which will therefore modify the results of the hormonal reading.

The authors pay little attention to the hormonal reading of these smears, and we believe that the better smear is the one that still provides information concerning the hormonal, malignant and inflammatory conditions of the female genital tract.

Using the cervical smear alone, many cases of carcinoma of the uterine corpus and some cases of endocervical malignancies will be missed. For this reason, we recommend the technique of taking three samples: vaginal, cervical and endocervical.

J. PAUL PUNDEL, Luxembourg, Luxembourg:

For 12 years I have used only a wooden tongue blade for the collection of vaginal and cervical smears. I agree with Richardson that if offers the most satisfactory technique feasible in the physician's office.

The only disadvantage of cervical scrapings is their low accuracy in detection of uterine cancers. In suspicious cases, one could take additional aspiration smears from the endocervix or from the endometrial cavity. But I think that for the detection of corpus cancer one should not rely too much upon cytology and suspicious cases should be investigated by curettage even if the smears are negative.

## CLOSING REMARKS

FRIEDRICH BAJARDI:

As already mentioned, in our hands the cytological smear is always prepared after the colposcopical examination. That is in my opinion the reason for the difference of our own experiences as compared to those reported by Hunter. Colposcopy is necessarily preceded by treatment of the ectocervix with 3% acetic acid, which results in the ectocervix being cleansed of mucus and other contamination. Smears taken after this procedure (by the scraping method) contain, in the majority of cases, cells which have been scraped from the intact epithelium. These scraped off cells are, as a rule, better preserved than those which have exfoliated spontaneously from the epithelial surface and there are hardly any disturbing contaminations of the smears with mucus.

Diagnostic accuracy of detecting endometrial adenocarcinoma is somewhat reduced by the cleansing of the ectocervix with acetic acid for colposcopy. As mentioned above, the diagnostic accuracy amounted only to about 30% in our material. However, like Pundel, we do not rely, in regard to the detection of adenocarcinoma of the endometrium, on a negative cytological smear but only upon the results of curettage.

Finally, concerning the doubts uttered by Hunter in regard to the hyperplastic basal cells, I feel that I have not been understood correctly. The picture of an epithelium, the upper third of which is composed of almost normal cells and the lower two-thirds undistinguished histomorphologically from a carcinomatous epithelium, is generally known. In those "atypical basal cell hyperplasias" the spontaneously exfoliated, normal superficial cells will not give rise to cytological suspicion. If one succeeds, however, in reaching with the scraping method the cytomorphologically atypical cells from the deeper layers, than a positive or suspicious cytological reading may result.

HOWARD L. RICHARDSON:

To Hunter:

The use of the glycol-acetic fixative formula, as given in this series of articles, is advantageous inasmuch as it destroys the red blood cells and penetrates mucus, whereas the alcohol-ether preparation congeals the erythrocytes and mucus in thick smears. The acetic acid hemolyzes the erythrocytes, and the slow penetration of the mucus by the glycol derivatives allows the stains to penetrate the clusters in depth. In our experience the screening time is shortened by the abundance of cell material with the glycol fixative.

# MATERIAL OBTAINED BY TWO TECHNIQUES: (a) VAGINAL SMEARS AND (b) CERVICAL SMEARS

JAMES HENRY FERGUSON AND
MARTIN H. MATZ*
Miami, Florida, U.S.A.

Several investigators have attempted to evaluate the usefulness of the vaginal tampon smear as a means of mass screening in cervical cancer detection. Most of these studies, however, have dealt with the results obtained by vaginal tampon only, and the subsequent tissue examinations which were indicated by these smears. It was felt by the authors that a retrospective study, which examined a group of patients, all with histologically proved cervical carcinoma, and all having had both vaginal tampon smears made from surface cell scrapings with a wooden spatula, would be of some value in assessing the relative accuracy of these two methods.

Accordingly, a group of 52 women who met these criteria was studied. By histologic examination of tissue specimens, 33 of these patients had invasive carcinoma of the cervix, while the remaining 19 patients had carcinoma in situ of the cervix. All of this group had smears made by the surface cell scraping technique with a wooden spatula and also smears made by the vaginal tampon technique prior to the establishment of a tissue diagnosis of cancer. The tampons were left in place a minimum of 4 hours and a maximum of 24 hours. All 3 studies—the tampon smear, the spatula smear, and the acquisition of tissue for microscopic examination—encompassed no longer than an eight-week period, the average being 3 to 4 weeks. The smears were stained by the routine Papanicolaou method and were rated according to Papanicolaou's classification.

The results were as follows:

1. In the 33 patients with invasive cervical cancer, the spatula method reported 28 cases as Class IV or V, and 3 cases as Class III. In only 2 cases was the report negative. This represents an error of 6%. In these same 33 patients, the tampon smear reported 22 cases as Class IV or V and 2 cases as Class III; there were 9 negative smears, an error of 27%.
2. In the 19 patients with cervical carcinoma in situ, the spatula revealed 13 Class IV or V smears, 5 Class III smears, and one negative smear, an error of 5.3%. The tampon in this group showed 4 smears which were Class IV or V, 7 smears were Class III, and 8 negative smears, an error of 42%.
3. The combined results for both groups were: Spatula smear was inaccurate in 3 cases out of 52; 5.8% error. Tampon smear was inaccurate in 17 cases out of 52; 33% error.
4. As an adjunct with atypia of the cervix 9 patients with atypia of the cervix were studied. All nine of these had positive smears with the spatula technique, while only one of these had a positive tampon smear.

From this study we believe that the tampon smear, while it may have value in patients who will not submit to pelvic examination, does not approach the degree of accuracy of the smear made by the surface cell scraping technique. Further studies are at present under way to enlarge this series for continued evaluation of the problem.

EMMERICH von HAAM AND
EDWARD M. MILLER
Columbus, Ohio, U.S.A.

This is the method recommended by us to physicians who have adopted the Papanicolaou smear as routine examination for their patient. It is not recommended to gynecologists only but to general practitioners, and we strongly emphasize that both procedures should be performed by the physician using a speculum and visualizing the areas from which the smears are taken. The great advantage of this method lies in its greater accuracy, as we pointed out in a previous part of this symposium. Three times as many malignant tumors were missed in the large cancer survey by using only the vaginal aspiration technique without visualization of the uterine cervix. In comparing the vaginal and cervical smears taken by physicians at the same time, we find that cervical smears usually are more helpful for the recognition of malignant

*From the Department of Obstetrics-Gynecology, University of Miami School of Medicine and Jackson Memorial Hospital, Miami, Florida.

cells but are less helpful for the hormonal evaluation of the patient, for the recognition of Trichomonas Vaginalis and other parasites, and for the correct evaluation of inflammatory changes. For the hormonal evaluation we prefer a vaginal smear taken from the wall of the middle third of the vagina, but an aspiration of the vaginal pool will also give us a better picture of the hormonal status than a cervical smear, since the cervical epithelium does not always accurately reflect the hormonal changes. Erosion cells and endocervical cells are disturbing factors, and the fact that cells appear more in clumps than singly often will make the determination of the Karyopyknotic Index or the Eosinophilic Index impossible. We have found many cases in which the cells of a cervical smear show less estrogenic stimulation than the cells of its companion vaginal smear. Inflammatory changes are often grossly overemphasized in the cervical smear, which carries with it part of the cervical mucous plug with a high number of leukocytes. We always base our criteria of vaginitis or cervicitis on the inflammatory cells found in smears of the vaginal pool rather than on the number of leukocytes found in the cervical smear. Correct evaluation of the bacterial flora is also better on the vaginal smear, and Trichomonas Vaginalis are often found abundantly in the vaginal smear but are scarce in the cervical smear. As far as the accuracy of both smears in the diagnosis of early malignant lesions is concerned, we refer to the following Table in which corresponding vaginal and cervical smears are compared. In the majority of instances the diagnostic value of the cervical smear is superior to that of the vaginal smear, and in many instances a diagnosis of carcinoma in situ could not have been made on the vaginal smear alone. Only in the case of carcinoma of the endometrium do we find a greater accuracy with the vaginal smear, which accumulates malignant cells exfoliated from the endometrium in a higher concentration.

Table I

Discrepancies between Vaginal and Cervical Smears in 81 Cases of Uterine Cancer

| | | | |
|---|---|---|---|
| Vaginal smear | unsatisfactory<br>negative<br>suspicious | Cervical smear | suspicious . . 6<br>suspicious . . 47<br>positive . . . 24 |
| Cervical smear | unsatisfactory<br>negative<br>suspicious | Vaginal smear | suspicious . . 1<br>suspicious . . 1<br>positive . . . 2 |

In summary then, we feel that the use of the vaginal smear and the cervical smear together, taken by the physician after visualization of the cervix by speculum, represents the best method to date for the cytological examination of the female genital tract.


## JOSEPH S. SONG

Providence, Rhode Island, U.S.A.


Based on dual smear examination of 50,000 women screened, we have observed the following findings concerning material obtained by the two techniques. The advantages in examining vaginal smears are:

1. Lesser degree of cellular overlapping and even distribution of cells.
2. Smaller number of endocervical cells which require time-consuming examination.
3. Relatively easy technique for obtaining smear material.

The disadvantages in examining vaginal smears lie in:

1. Comparatively small number of interspersed positive cells so that the entire smear field has to be thoroughly examined.
2. Insufficient material for examination, especially when the smear is made from elderly women.

Cervical scraping smears collected by means of a wooden spatula have the following advantages:

1. Contain sufficient amount of material to be examined as compared with vaginal smears.
2. Presence of relatively large number of positive cells in the group.

The disadvantages in examining cervical smears are:

1. The presence of a great number of endocervical cells which require more time and experience for adequate interpretations.
2. Much more cellular overlapping than vaginal smears.
3. Considerable loss of cellular material from the smears during staining procedure.

We have been using the completely frosted slide in order to obtain smears with sufficient amount of cellular material. When the vaginal smears are made on the rough surface of the frosted slides, the slides usually contain sufficient material for examination. This slide is particularly useful in obtaining vaginal smears from elderly females who usually have scanty material to be aspirated. To avoid much cellular overlapping in cervical scraping smears, we have found that the regular smooth slide with a frosted tip is suitable for making thin, even smears.

In our series, we have found that both types of smears offer similar diagnostic value in detection of fundal carcinoma.


## STANLEY WAY AND NANCY DAWSON

Newcastle upon Tyne, England, U.K.


We have, almost since starting cytological diagnosis, asked for both a scrape and aspiration smear in every patient. It has been our impression that as far as diagnosis was concerned the scrape smear was probably more reliable than the aspiration smear and, even more important, easier and quicker to diagnose. Clearly any method in diagnostic cytology which saves time reliably is worthy of consideration. Recently we investigated this problem more closely.

### Material

The material consisted of 114 positive cytological diagnoses made in this laboratory in the latter half (July-December) of 1958. Seventy-two of these cases were diagnosed clinically as carcinoma of the cervix. Forty-two cases were unsuspected and on histological examination 24 of these proved to be carcinomas in situ while 18 showed microinvasion.

### Method

All the slides were fully screened and reported as positive or negative. One hundred cells on each slide were selected at random and divided into normal and malignant. For the purpose of this investigation only definitely malignant cells were classed as such. Dubious

cells were regarded as normal.

In one case a complete count of every cell on the slide of both aspiration and scrape smears was performed and the cells divided into malignant and benign. In the counts on this smear from 100 cells selected at random the results were:

| | Aspiration Smear | Scrape Smear |
|---|---|---|
| Benign | 100% | 76% |
| Malignant | 0% | 24% |

From the total differential counts on the entire smears the results were:

| | Aspiration Smear | Scrape Smear |
|---|---|---|
| Total Cells | 25,741 | 12,607 |
| Benign | 99.8% | 83.4% |
| Malignant | 0.2% | 16.6% |

So time consuming was this that only one smear was dealt with in this way.

The cases were then separated into clinical carcinomas and pick up cases.

### Clinical Carinomas

On complete screening of the scrape smear, 72 cases (100%) were positive whereas in the aspiration smear 66 (91.7%) were positive.

On counts of 100 cells, 66 scrape smears showed a higher percentage of malignant cells than the aspiration smears. In one case both smears showed an equal percentage while in five cases the aspiration smear showed a larger percentage.

The average percentage of malignant cells in the aspiration smears was 19.28 while in the scrape smears it was 23.37. The difference is obviously small and not significant.

### Pick-up Cases

On complete screening of the scrape smear 42 cases (100%) were positive whereas in the aspiration smear 28 (66.6%) were positive.

On a count of 100 cells the scrape smear contained a greater percentage of malignant cells than the aspiration smear in 39 cases (93%) while in three cases the reverse was obtained.

The average percentage of malignant cells in the aspiration smear of all pick-up cases was 1.79 and in the scrape smears 10.74.

The cases were then divided into microinvasive and non-invasive.

### Microinvasive

In these cases the scrape smear contained a greater number of malignant cells in 15 out of 18 cases (83%) and three of the aspiration smears were negative on total screening (17%).

### Carcinoma in situ

Of the 24 cases the aspiration smear was negative in 11 (46%) and the scrape smear contained a greater percentage of malignant cells in every case.

The scattergram shows the distribution of the individual cases.

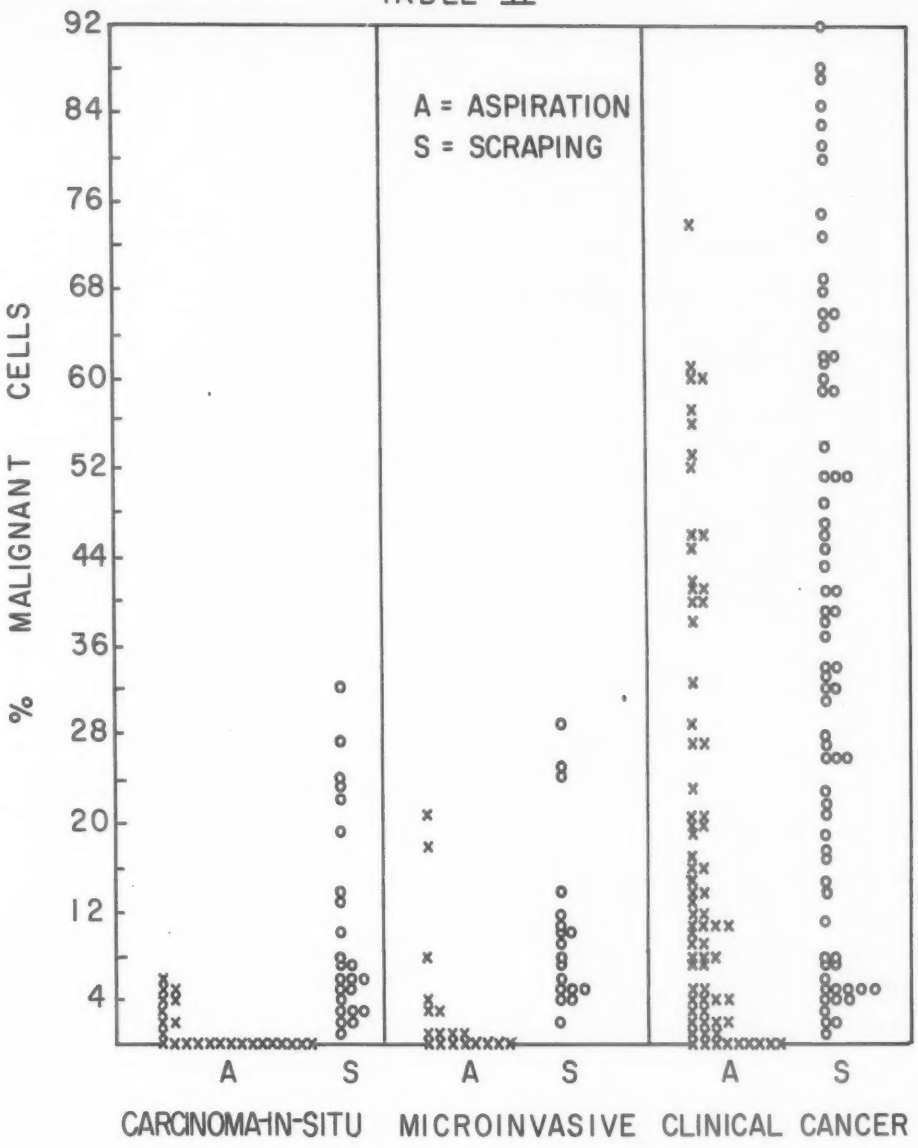
### Conclusions

1. Both aspiration and scrape smears show a high degree of reliability.
2. In non-clinical cancer, exfoliation is less than in clinical cancer and this is reflected in both types of smear.
3. In both clinical and non-clinical cancer the scrape smear usually shows a higher percentage of malignant cells per 100 cell population than the aspiration smear.
4. In clinical cancer the differences between scrape and aspiration smear is slight, being reflected by a factor of only 1.2:1.
5. In non-clinical lesions as a whole the differences between scrape and aspiration smears is more marked, being reflected by a factor of 6:1.
6. In carcinoma in situ the factor is 10:1 in favor of the scrape smear and in microinvasive cancer it is 5:1.
7. Forty-six per cent of aspiration smears in carcinoma in situ were negative on total screening and 17% in microinvasive cancer.
8. We conclude that in non-clinical cancer the scrape smear is more reliable than the aspiration smear and that in clinical cancer it shows a slight advantage.
9. A higher percentage of cells is found in scrape smears in both clinical and non-clinical cancer. This makes for ease of diagnosis and saving of time which is a very important consideration in view of the great value of cytological diagnosis and the shortage, the world over, of adequately trained personnel.

Table I

| Pick up cases | Total Cases | Screening | | | | Counting | | |
|---|---|---|---|---|---|---|---|---|
| | | Scrape | | Aspiration | | Scrape greater % malignant cells than aspiration | Aspiration greater % malignant cells than scrape | Both smears equal |
| | | + | -v | + | -v | | | |
| a) Microinvasive | 18 | 18 | 0 | 15 | 3 (17%) | 15 | 3 | 0 |
| b) Carcinoma in situ | 24 | 24 | 0 | 13 | 11 (46%) | 24 | 0 | 0 |
| (Average % of malignant cells per smear = scrape: 10.74, aspiration: 1.79) | | | | | | | | |
| Clinical Cancer | 72 | 72 | 0 | 66 | 6 (8%) | 66 | 5 | 1 |
| (Average % of malignant cells per smear = scrape: 23.37, aspiration: 19.28) | | | | | | | | |

TABLE II




## DISCUSSION

RUTH M. GRAHAM, Buffalo, New York, U. S. A.:

Whether a cytologist prefers vaginal smears or cervical scrapings is a matter of personal preference. However, I would not like to have the readers of this Symposium come to the conclusion that the vaginal smears are not adequate for the cytologic diagnosis of uterine cancer. It might be remembered that in Papanicolaou's original monograph (1) he states "In the entire group of 127 patients with demonstrable lesions of the cervix, failure to detect the malignant cells in the vaginal smears occurred four times." In a paper confirming Papanicolaou's observation, cancer cells were found in 45 of 46 cancer of the cervix patients. I am certain that if the accuracy figures quoted in these two papers had been similar to those quoted in this Symposium for vaginal smears there would have been little interest in the cytologic diagnosis of cancer of the uterus. Our present figures are much the same as the original ones of sixteen years ago as may be seen in the following Table.

Vaginal smears are highly effective. The limita-

Table I

| Vaginal Smear | Carcinoma of the Cervix | In Situ | Corpus |
|---|---|---|---|
| Positive | 284 | 50 | 46 |
| Doubtful | 5 | 2 | 1 |
| Negative | 7 | 4 | 3 |

tions of the method are largely requirements of good technical preparation and diligent screening by able personnel.

Bibliography


1. Papanicolaou, George N. and Traut, Herbert, F.: Diagnosis of Uterine Cancer by the Vaginal Smear, The Commonwealth Fund, New York, 1943.
2. Meigs, J. V. et al.: The Value of the Vaginal Smear in the Diagnosis of Uterine Cancer, Surg. Gynec. and Obst. 77:449, 1943.


PIERRE J. HAOUR, Lyon, Rhône, France:

Way and Nancy Dawson are to be congratulated for their important work on this question as they bring definite proof concerning the greater accuracy of cervical scraping in the diagnosis of cervical carcinoma and especially of non-clinical tumors, as compared with vaginal aspiration. Ferguson also brings significant results.

It is evident that cervical scraping gives a larger specimen, but the elements obtained are not only exfoliated cells, but cells removed, by the procedure, from deeper layers.

From Way and Nancy Dawson's work it does not appear that it is necessary to associate both methods, and vaginal aspiration must be considered only in certain circumstances which do not permit the introduction of a speculum.

In 256 cases of cervical cancer, using both cervical scrapings and smears obtained with a spatula from the vaginal pool, our results are similar to Ferguson's with an error of 5%.

ALEXANDER MEISELS, México, D.F., México:

The foregoing papers really discuss five different techniques of obtaining material for routine cytological examination:

1. Vaginal tampons.
2. Vaginal smears obtained by aspiration of the posterior vaginal pool.
3. Vaginal smears obtained from the lateral wall of the vagina.
4. Cervical smears obtained by aspiration.
5. Cervical scrapings.

1. The results obtained by Ferguson and Matz once more show that the vaginal tampon method has only very limited possibilities. Even if at first it seemed a good method for mass-screening, its unreliability, as shown in this paper, should prove reason enough to abandon its use completely.
2. Although this method, when used, alone, accounts for a certain number of false negative reports, it should never be omitted in a routine study, since it gives a very good over-all picture of the genital tract. In some cases, this is the only smear that will show cells exfoliated from an adenocarcinoma of the endometrium, and routine cytology cannot limit its usefulness to the detection of cervical carcinomas alone, but should try to obtain as much information as possible on the condition of the whole genital tract. But although this smear probably represents the most useful single method, it seems insufficient, if used alone, to detect some cases of carcinoma, and to give an adequate picture of the hormonal level.
3. The smear obtained from the lateral wall of the vagina (I prefer the upper third) is the best method for obtaining a cytological impression of the hormonal levels. It is a very reliable method for this purpose only. Hormonal evaluations done on smears from other sites may show excessive variations due to non-hormonal factors. This is especially true for smears taken from the vaginal pool and from the cervix. Also, the smear from the lateral wall of the vagina provides the possibility of comparing results in different cases, because the cells examined always have the same known origin, in contrast to cells collected from the vaginal pool.
4. This smear gives a very clear and rich picture, but has the disadvantage that sometimes there is not enough material to be aspirated. In our experience, the cervical aspiration yields smears that compare very well with the cervical scraping method.
5. This method is very reliable, and can be used in almost every case. I agree that it should not be omitted for routine examinations.

Smears 2, 3, 4 and 5 should always be obtained through a speculum. Blind aspiration is unreliable even in experienced hands.

This leaves us with three smears that should be done routinely: aspiration from the vaginal pool, smear from the lateral vaginal wall, and cervical smear, either by aspiration or by scraping. For routine detection of cervical cancer, the first and the last are musts, as pointed out by von Haam and Miller. The smear from the lateral wall of the vagina should be included, whenever possible, in order to obtain valuable data on the hormonal levels of the patient.

LUIS MONTALVO-RUIZ, Madrid, Spain:

We agree with the authors that smears from scrapings are superior to aspirated smears in the diagnosis of cervical cancer. When we used the aspiration technique with the Papanicolaou pipette, between 1948 and 1954, our error in the diagnosis of cervical cancer was 18% false negatives, but since 1955 we have been using the scraping procedure and now this figure has been reduced to 4% false negatives. The above results were given by us in Malaga, in December, 1956, during the meeting of Spanish gynecologists (1).

We do not completely agree with Stanley's second conclusion. We have found a greater percentage of malignant cells in carcinoma in situ than in invasive carcinoma and a greater proportion of positive cases in carcinoma in situ.

Bibliography


1. Montalvo, L.: Toko-Ginecologia Practica. 158:19, 1958.


HANNAH PETERS, Copenhagen, Denmark:

I would like to underline the statement von Haam and Miller made: the importance of visualization of the area from which the smear is taken. This is of impor-

tance in the preparation of smears taken for cancer readings as well as for hormonal evaluation. I cannot quite agree with the opinion that the cervical epithelium does not always accurately reflect the hormonal changes. Smears taken from the ectocervix do in fact give certain information of the hormonal status, which the vaginal smear alone is not always able to convey. The epithelium of the ectocervix responds more sluggishly to estrogen stimulation than does the vaginal lining. This becomes useful in those cases in which estrogen stimulation causes the vaginal epithelium to fully mature and results in a mature vaginal smear but is not high enough to stimulate the cervical epithelium. In such a case the cervical smear will be of the intermediate or even the atrophic type. The fact that the two smears differ, can therefore convey more extensive information on the hormonal status than the vaginal smear alone. However, in this case, as in cancer diagnosis, the smear should be taken under direct vision.

WOLFGANG WALZ, Heidenheim, Brenz, Germany:

All the workers agree that taking the cervical smear with aid of a spatula yields the best results in regard to early cancer detection, as compared to the aspiration method. We have checked this question on 500 patients by controlling the cytological findings with the colpomicroscope. We also found that taking the cervical smear with a wooden spatula is the safest method for early cancer detection.

This may be explained by the fact that at the time of aspiration the normally exfoliated cells have been in the vaginal pool for widely varying periods of time. For this reason there is a wide gap between the cytological findings and the results of the colpomicroscopical examination. Therefore, even for the cytohormonal evaluation one should obtain the vaginal specimen not by aspiration but by scraping of the epithelial surface, in order to get fresh and unchanged cellular material. Where cancer detection is concerned, obtaining the specimen from the cervical surface with a spatula is by far preferable to the vaginal smear.

## CLOSING REMARKS

JAMES HENRY FERGUSON:

I cannot agree with the decision of Meisels that the vaginal tampon should be abandoned completely. There is a possibility that the screening tampon can be made a more sensitive instrument by changes in its size and structure. Even if the tampon never meets the accuracy of other methods of cell collection, a smear from an asymptomatic woman made by a tampon is better than no smear at all. A future for the tampon may lie in the screening of groups of women who cannot or will not have smears made by better techniques.

STANLEY WAY and NANCY DAWSON:

Ruth Graham's good technical preparation, diligent screening and the able personnel in her laboratory have long been the admiration of both of us who have learned much from her and her assistants in our visits to her laboratories. We would say at once that conditions in our laboratory are different. Most of our smears are prepared in local hospitals over a wide region and frankly we do better with scrape smears. Thus conditions under which we work are different from those under which Ruth Graham works. One of us (S.W.) has a sneaking feeling that if he could persuade Ruth Graham to use scrape smears her results would be even better, in other words they would almost achieve perfection.

We would like to point out to Haour that we did not wish to give the impression that we believe the aspiration smear should be omitted.

Our contribution to this Symposium concerned only the diagnosis of cervical carcinoma. We have evidence (not quoted in our paper) to show that in the diagnosis of corpus cancer the reverse is true and that the aspiration smear is the most reliable. Further, in estimating such aspects as cornification etc., in both malignant and benign cases we believe that only naturally exfoliated cells should be studied. Cells obtained by scraping do not fall into this category. We should like most emphatically to disagree with Hannah Peters that visualization is necessary for accurate smears in cancer diagnosis in relation to aspiration smears.

One of us (S.W.) is a gynecologist of great experience, trained and practised at digital vaginal examination (which of course is performed blind) and he has, in fact, been doing this day in and day out for longer than the other of us (N.D.) has been alive.

As a result he can tell accurately exactly where his pipette is when inserted blind. Moreover he is prepared to go anywhere if challenged (at the challenger's expense) to prove this.

Despite this egotistical profession of vaginal "legere de main" he is forced to confess that of the aspiration smears taken by the two of thus those of N.D. are the more accurate. N.D.'s scrape smears are not as good because she is not so skilled at visualizing the cervix, which all goes to show how true are the concluding discussion remarks of Ruth Graham.

We have expressed our belief as we see it in the light of our experience but we would be the last to carry our desire for scrape smears, in a search for perfection, to extremes, especially where such are difficult and undesirable to obtain, but in such cases would remember always Shakespeare's Sixty-Sixth Sonnet which might have said:-

A maiden virtue rudely strumpeted
And right perfection wrongfully disgraced
. . . and folly—doctor-like—controlling skill
. . . Tired with all these, from these I would be gone
Save that to die, I leave cytology alone.

# MATERIAL OBTAINED BY THREE TECHNIQUES: (a) VAGINAL SMEARS, (b) CERVICAL SMEARS AND (c) ENDOCERVICAL SMEARS

WERNER BICKENBACH AND
HANS-JÜRGEN SOOST
Munich, Germany

When several smears are taken simultaneously from a patient with a cervical carcinoma, the number of atypical cells can vary greatly with the site from which the smear was taken.

Figure 1 shows the results in percentages of cells, either suspicious of carcinoma, doubtful or normal, after counting a total of 30,400 cells in 98 cytological smears. Smears from the ectocervix usually show the greatest number of atypical cells in good condition. Smears from the cervical canal contain very many doubtful cells in addition to atypical cells. It may be very difficult to arrive at an opinion about an endocervical smear since the autolytic cell changes occurring in benign conditions, such as cervicitis, can create a very doubtful picture. The posterior fornix shows the least number of atypical cells.

After taking several smears simultaneously from different sites in 25 patients with cervical carcinoma, the results are depicted in Figure 2.

It is true, however, that the largest number of suspicious cells are not necessarily found in every case at the ectocervix. In the presence of a cervical carcinoma situated higher, endocervical smears frequently show a larger number of atypical cells, while in more advanced carcinoma diagnosis is often easiest when the smear is taken from the posterior fornix.

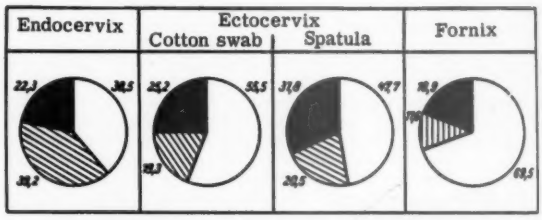



Fig. 1. Percentage figures for suspicious ■, doubtful ▨, and normal □ cells found in smears taken from different sites, determined by counting 30,400 cells in 98 cytological smears.

Optimal cytological results can be expected only when three smears are taken simultaneously: from the ectocervix with a spatula, from the cervical canal and from the posterior fornix with cotton swabs. This technique is imperative for all doubtful cases.

In routine diagnosis it is impossible to take three smears in every case without overburdening the clinician and without overloading the capacity of the cyto-

| Site from which Smear was taken | No. of Smears | Pap. IV & V Suspicious | Pap. III Doubtful | Pap. I & II Not Suspicious |
|---|---|---|---|---|
| Endocervix | 22 | 17 | 5 | - |
| Cervix (with cotton swab) | 31 | 30 | 1 | - |
| Cervix (with spatula) | 15 | 14 | 1 | - |
| Fornix | 30 | 21 | 5 | 4 |
| Lateral vaginal wall | 6 | 1 | 2 | 3 |

Fig. 2. 104 smears were taken from 25 cases of cervical carcinoma; the smears having been taken from different sites as specified. It can be seen that the relative certainty of diagnosis depends upon the site from which the smear is taken.

logical laboratory. It is, therefore, particularly important that smears be taken with special care from the optimal site in all cases in which only a single smear is taken. For this purpose we consider the ectocervix the site of choice.

In our opinion the 5-10% (approximately) false-negative findings, cited in most statistics on the cytodiagnosis of cervical cancer, appear to be due to avoidable mistakes in the taking of the smears. Therefore, one cannot overemphasize, to the clinician, the importance of correct technique in establishing a cytological diagnosis with certainty.

Bibliography

1. Soost, H. J.: Krebsarzt 13:408, 1958.

JEAN A. de BRUX

Paris, France

Our aim in this topic has been, not to analyze the different cells collected by the three techniques, but to determine which of the three varieties of smears we had found most useful in diagnosing a lesion. We have arbitrarily taken 122 cases of squamoid carcinoma of the cervix and 31 cases of endometrial adenocarcinoma. All cases were confirmed histologically. We have evaluated each of the smears, taking into account the number of abnormal elements collected by the different methods. We have chosen the following code:

0 = no cancerous cells
\+ = rare cancerous cells
++ = rather numerous cancerous cells
+++ = numerous cancerous cells
++++ = very numerous cancerous cells.

Of 60 cases of squamoid carcinoma of the cervix, we have compared the three smears and have found:

in 1 case, the highest number of cancerous elements was on the vaginal smear;
in 42 cases, the highest number was on the cervical smear;
in 12 cases, the highest number was on the endocervical smear;
in 5 cases, the cervical and endocervical smears showed the same number of cancerous cells.

Of 30 cases of endometrial adenocarcinoma, we found:

8 cases in which the vaginal smear alone gave the diagnosis;
12 cases in which the vaginal and cervical smear had the same aspect;
1 case in which the cervical smear alone afforded the diagnosis;
4 cases in which the cervical and endocervical smears had the same number of cancerous cells;
1 case in which the endocervical smear alone gave the diagnosis;
4 cases in which the intra-uterine smear alone gave the diagnosis.

Conclusion

The diagnosis of squamoid carcinoma of the cervix is given with the greatest frequency by the cervical and endocervical smears.

The desquamated elements of an endometrial adenocarcinoma are found in greatest number on the vaginal and cervical smears.

But, given the fact that even in a voluntarily restricted statistical compilation, there are cases in which the diagnosis was made on one of the smears to the exclusion of the other two, we deem that all the methods of

Squamoid carcinoma of the cervix - 122 cases:

| | Vaginal Smears | Cervical Smears | Endocervical Smears |
|---|---|---|---|
| 0 | 22 | 0 | 4 |
| + | 24 | 4 | 4 |
| ++ | 25 | 31 | 11 |
| +++ | 20 | 39 | 18 |
| ++++ | 8 | 35 | 19 |
| Total cases diagnosed: | 77 out of 99 | 109 out of 109 | 52 out of 56 |

Carcinoma of the corpus uteri - 31 cases:

| | Vaginal Smears | Cervical Smears | Endocervical Smears | Intra-uterine Smears |
|---|---|---|---|---|
| 0 | 8 | 4 | 6 | 1 |
| + | 0 | 3 | 3 | 0 |
| ++ | 3 | 7 | 2 | 0 |
| +++ | 7 | 6 | 4 | 3 |
| ++++ | 6 | 6 | 2 | 1 |
| Total cases diagnosed: | 16 out of 24 | 22 out of 26 | 11 out of 17 | 4 out of 5 |

sampling—vaginal, cervical, endocervical and intra-uterine—should be employed for the detection of a cancer.

## EMMERICH von HAAM
### Columbus, Ohio, U.S.A.

The examination of endocervical smears has been recommended for the discovery of endocervical and endometrial malignancies. While cells from tumors originating in these sites do exfoliate into the vagina, where they can be detected in small clumps after painstaking examination of smears prepared from the vaginal pool (1), they can be found in greater numbers and in a better state of preservation in smears prepared from endocervical aspirations. The endocervical aspiration technique differs from the endometrial technique in that the aspirating pipette should never pass beyond the internal os of the cervix. Thus trauma to the uterine cavity, including the possible disturbance of an early pregnancy, which Terzano (2) cites as a disadvantage of the routine use of endometrial aspirations, can be avoided. The endocervical aspirations also rarely contain much blood, which makes the endometrial aspiration often such a difficult problem to the examining cytotechnologist. In multiparas with lacerated external cervical os the endocervical aspiration can often be performed without additional dilatation of the cervical canal by introduction of the small laryngeal cannula recommended by Papanicolaou (3) or the pipette of McClure recommended by Smolka (4). In virgins or nulliparas slight dilatation of the cervix is usually necessary. Smears prepared from the cervical aspiration will consist mostly of endocervical and endometrial cells, although in multiparas with distorted external os many squamous epithelial cells can be found. An endocervical smear will lend itself poorly to evaluation of the estrus of the patient and will not be useful for the recognition of cervical erosions, cervical leukoplakia or Trichomonas infection. However, it will reflect well the condition known as chronic endocervicitis and will permit a better study of endometrial cells, which can be differentiated from endocervical cells by their slender appearance and the smaller and usually darker nuclei. As far as recognition of malignancies is concerned, endocervical aspirations have proven less satisfactory for the recognition of carcinomas in situ or invasive carcinoma of the cervix but have their value in the recognition of endometrial carcinoma. Thus by combining the three techniques, the number of correct diagnoses of malignant tumors can still be increased over the accuracy established by the vaginal aspiration and cervical smear technique.

The disadvantages of using all three techniques in the routine cytological examination of female patients are: 1) that more instrumentation is needed, thus making the examination more time-consuming, and 2) that sometimes slight pain cannot be avoided during the process of dilatation of the cervical canal. The cytotechnologist also will have to spend more time on a specific case, since now a minimum of 3, and possibly 6, slides must be examined from each patient.

In our relatively small series of 439A cases (Table 1) in which the private patients of three gynecologists were examined by this technique, endocervical aspiration proved twice as accurate in the recognition of endometrial carcinomas, but the total accuracy of our series was only increased from 95.2 to 96%.

We feel therefore that the endocervical aspiration should not be used routinely in the cytological examination of normal, menstruating women, but is recommended only for patients who are in menopause and who are suspected of having carcinoma of the endometrium. It may also be helpful in cases where the surface of the cervix is necrotic or scarred, such as we sometimes find in advanced invasive carcinomas after x-ray therapy.

Bibliography


1. Berg, J. W. and Durfee, G. R.: Cancer 11:158, 1953.
2. Terzano, G.: ACTA CYTOLOGICA 2:567-68, 1958.
3. Papanicolaou, G. N.: Atlas of Exfoliative Cytology. Cambridge, Mass., 1956, Commonwealth Fund.
4. Smolka, H. and Soost, H. J.: Grundriss and Atlas der Gynäkologischen Cytodiagnostik. Stuttgart, 1956, Georg Thieme.


## LUIS MONTALVO-RUIZ
### Madrid, Spain

Without doubt this method is the most complete for cytologic study, whether of malignant or benign cases. We will miss very few carcinomas by taking these three samples. Besides, they have the advantage of giving information about the hormonal status of the patient and the bacterial and parasitic status of her vagina and cervix.

A. Vaginal smear: We obtain the vaginal smear by aspirating with a pipette. We spoke of its advantages elsewhere in this Symposium. We do not use the platinum loop, the "sponge biopsy" or the direct in-

Table I

4394 Cases Examined by All Three Techniques

| Type of Lesion | No. Cases | Diagnosis Made by | | | Total Accuracy % |
|---|---|---|---|---|---|
| | | Vaginal Aspiration | Cervical Scraping | Endocervical Aspiration | |
| Normal cervix | 1580 | 1496 | 1506 | 1487 | 94 |
| Cervical erosion | 1650 | 1241 | 1476 | 1061 | 89 |
| Endocervicitis | 1137 | 761 | 976 | 1072 | 94 |
| False positives | 186 | 21 | 76 | 15 | 4.2 |
| Carcinoma in situ | 14 | 12 | 14 | 10 | 100 |
| Invasive carcinoma | 9 | 7 | 8 | 7 | 89 |
| Carcinoma of endometrium | 4 | 2 | 2 | 4 | 100 |

travaginal smear on the slide as recommended by Hyams, Hyams and Wainess (2), in scanty secretion cases.

B. Cervical smear: We obtain the cervical smear by scraping superficially and deeply with a spatula. We do not use the brush as recommended by Pund et al. (3).

C. Endocervical smear: We obtain the material with a cotton swab soaked in saline solution. When the cervical secretion is scanty it is possible to put a plastic spoon in the cervix for 24 hours as recommended by Doyle (1).

All the samples must be taken under usual control, after a vaginal speculum is put in place, so we can obtain material from the suspicious zones. In virgins, a virgin's speculum is inserted and the material is obtained with a pipette or a cotton swab.

VAGINAL SMEAR (Aspiration)

| Advantages | Disadvantages |
|---|---|
| It is easily performed and can be repeated. Good for serial investigations. It gives the hormonal, bacterial or inflammatory status. | Scanty endocervical and endometrial cells present. Insufficient for cancer diagnosis |

CERVICAL SMEAR (Scraping)

| Advantages | Disadvantages |
|---|---|
| It is easily performed. One can obtain material from the suspicious zones. More atypias are seen than with the aspiration method. Deeper epithelial cells are obtained. | Insufficient for hormonal reading. It may harm the epithelium or cause bleeding of friable epitheliums. Mucus makes the smear hard and thick. |

ENDOCERVICAL SMEAR (Cotton swab)

| Advantages | Disadvantages |
|---|---|
| There are an increased number of endocervical and endometrial cells. It gives a more accurate diagnosis of carcinoma of the cervix and endometrium. | Too much mucus. There is the possibility of missing a carcinoma of the vaginal portion. Insufficient for hormonal reading. |

We take the smears either on three slides or on one with three divisions (4). The latter procedure is less time consuming and more economical. It is advisable in big populations. However, it has the disadvantage that while preparing one division of the slide another division is drying. That is why there are some smears found with pseudo-eosinophilia. In private practice we take the vaginal smear on one slide and the cervical and endocervical on another one, so we avoid the aforesaid trouble. We are interested in keeping the correct staining reaction in order to be able to make the correct hormonal reading.

Bibliography


1. Doyle, J. B.: New Engl. J. Med. 243:121, 1950.
2. Hyams, M. M., Hyams, R. G. and Wainess, E. E.: Am. J. Obst. Gyn. 59:445, 1950.
3. Pund, E. R., Nieburgs, H. E. and South, M. J.: 43: 384, 1950.
4. Wied, G. L.: Acta Gin. 7:563, 1957.


## DISCUSSION

FRIEDRICH BAJARDI, Graz, Austria:

It has been demonstrated statistically by the above authors that we can expect an increased cytological accuracy by applying three different techniques. The routine application of this enlarged cytological investigation is particularly desirable because of the greater detection of carcinoma of the endometrium.

However, it is doubtful whether or not it would be practical to provide the additional labor and expense necessary for the application of this wider technique. This applies not only to my own clinic but in all the clinics throughout the world. In our own work we examine in each patient at least one smear, irrespective of the clinical symptoms. Like Bickenbach and Soost and von Haam, we are unable to examine six or even three smears of each patient. Further smears are, therefore, only taken in particular cases, such as a suspected carcinoma of the endometrium in which the D and C, rendered imperative by this suspicion, cannot be performed for some special reason.

In such a selection of cases a proportion of those carcinomas of the endometrium which are still devoid of clinical symptoms will remain undetected. The number of these undetected cases will, however, be very small. As far as our own personnel and financial possibilities are concerned, an additional examination of at least 20,000 smears yearly, merely for the purpose of an earlier diagnosis of these few carcinomas, is not possible at present.

PIERRE HAOUR, Lyon, Rhône, France:

The figures given by the authors again justify the fact that the best procedure for collecting cancer cells is to obtain the smears from as near as possible to the site of the lesion. Therefore, cervical and endocervical smears should be preferred for detection of cervical cancer and intrauterine aspirations for adenocarcinoma of the uterus.

When one considers the statistics given by many participants to ACTA CYTOLOGICA, this appears to be the general opinion.

However, de Brux in his paper gives results in endometrial carcinoma which are more favorable to vaginal and endocervical smears than to intrauterine aspiration. The opinions of many on the accuracy of smears taken farther away from the site of cancer cell production are divergent: in the diagnosis of adenocarcinoma the accuracy of vaginal smears varies from 50% to 90% and for cervical smears from 45% to 85%.

Such differences depend not only on the variable experience of the cytologist but also on the way the smear is done and mostly on the staining technique.

For instance, I believe that it is difficult to find isolated cancer cells in the vaginal material, as was proposed by Ruth Graham, if the nuclei are over-stained. However, I agree that any type of smear, even those taken far from the lesions, may detect a cancer and we fully agree with de Brux, as was also stated by others in the Symposium on adenocarcinoma, that the three techniques should be used simultaneously as often as possible. This is not a problem for the gynecologist who per-

forms smears but we agree with von Haam that it may sometimes be difficult for the laboratory.

CONSTANTIN HEROVICI, Paris-Villejuif, Seine, France:

Having read the papers by de Brux, Bickenbach and Soost, von Haam and Montalvo Ruiz, one notices the perfect agreement of all the authors as to the advantage of obtaining cellular material from all levels of the female genital tract for detection of a subclinical cancer of the uterus. It seems logical, after a systematic examination, to decrease the possibilities of false negatives by increasing the number of sites from which the smear is taken. The problem is, of course, different when we deal with a visible cervical lesion. The cervical smear, in such instances, is the most valuable and richest in neoplastic cells. However, its importance in such cases is minimal, because if the lesion is visible it is up to a properly done biopsy to give the histological diagnosis. Finally there is the case of the lesion visible under hysterography in the uterine cavity. Here again the smear taken as close as possible to the lesion is the one that would naturally lead to the diagnosis.

We, together with Pundel, examined a series of 29 cases of carcinoma of the uterine corpus to determine at what level the cytological smear had the greatest chance of being negative. We have also compared the false negative cytological diagnoses with the false negative hysterographies and the histological examinations. The results are tabulated as follows:

In 29 cases of carcinoma of the uterine corpus:

| Method of Detection | | False Negative |
|---|---|---|
| Cytology | Vaginal smear | 12 |
| | Vaginal and cervical smear | 8 |
| | Vaginal, cervical and endocervical smear | 3 |
| | Vaginal, cervical, endocervical and intrauterine smear | 1 |
| Hysterography | | 4 |
| Histological examination | | 1 |

In conclusion, we take vaginal, cervical and endocervical smears in all those cases where the lesion is not visible, cervical smears when there is a cervical lesion which is visible, and endocervical smears when we suspect carcinoma of the uterine corpus.

Bibliography

1. Pundel, J. P. and Herovici, C.: Le Cytodiagnostic du Cancer Endometrial 5:22, 1956.

J. PAUL PUNDEL, Luxembourg, Luxembourg:

Nearly all statistics show clearly that the danger of missing a cancer of the uterus practically does not exist if the cell collection is as complete as possible. This requires at least three smears: a routine vaginal smear, a cervical scraping and an endocervical aspiration smear. The practical screening by cytology is, on the other hand, also a question of technical and financial possibilities. If these possibilities will not permit a complete examination by smears of all three types, I think that the above authors have brought out sufficient evidence that for cancer detection with a single smear a cervical scraping should be the method of choice. For technical reasons I have adopted the following routine. In asymptomatic patients showing no visible abnormalities of the cervix, either by colposcopy or by Schiller's test, I take only one cervical scraping. In all other patients I prefer a complete set of three smears, not only for cancer detection, but also for control of the biopsies.

## CLOSING REMARKS

WERNER BICKENBACH and HANS-JÜRGEN SOOST:

It was important to see that the cytologists agree in this question. It will now be another important problem to convey this opinion to the large number of gynecologists and practitioners. In our opinion, this is the best way to decrease the relative number of false negative cytological reports in the future.

JEAN A. de BRUX:

May I suggest to Bajardi that it is very easy to do a cervical smear on one edge of the slide, and on the other edge an endocervical smear. In this way, there is only one slide to examine, and two samplings; hence a greater chance of success.

For a correct evaluation of results, we must take into account the quality of the sampling, a factor which is equally valid for the pathologist.

Clinicians are prone to believe—most often in error—that they know how to correctly perform biopsies and smears. I imagine that everywhere there are certain clinicians whose results in smears and biopsies are generally excellent, and others for whom this is more rarely the case.

LUIS MONTALVO-RUIZ:

Bajardi states that it is not practical to take three smears from each patient because it raises the price and increases the work load for the detection of very few additional cases. If we use the same slide with divisions for the three smears, we do not raise the price because the same staining and the same slide as that used for one single smear is necessary. In addition, the three smears on one slide can give more diagnostic accuracy and better information with regard to hormonal and microbiological status.

Commenting on Haour's opinion that the different statements concerning diagnostic accuracy in adenocarcinoma (when the smears are taken far from the lesion) are probably due to the rare incidence of adenocarcinoma as compared with squamoid carcinoma explains why some authors are more lucky in their diagnosis than others. However, surely, when the number of cases increases the above discrepancies will disappear and be statistically significant.

We agree with Henrovici and Pundel.

# MATERIAL OBTAINED BY SPONGE BIOPSY

PETER STOLL
Heidelberg, Germany

Sponge-biopsy was inaugurated and described first by S. A. Gladstone in 1948. His experience was not restricted to gynecological carcinoma, but also included carcinoma of the rectum, stomach, lung, skin and oral mucosa. In the interim a few more papers were published in the gynecological field, to which I shall restrict my remarks.

It was the idea of Gladstone to touch the suspicious region with a sponge (Gelfoam No. 12, the Upjohn Co., Kalamazoo, Michigan) in order to suck cellular material into the spongy substance.

## I. Material

The applied gelatine sponge had as predecessor a gelatine sponge mostly used for hemostatic purposes. According to Taylor, during the past world war Harvard University carried out an extensive plasma fractionating program. There were sponges fabricated out of fibrin (Fibrin Foam) which, when saturated with thrombin, had a good hemostatic effect causing a minimum of tissue reactions. Near the end of the war two chemists, Correl and Wise, looked for a substitute for the fibrin and thereby discovered the gelatine sponge. They developed from the usual trade gelatine an absorbent sponge type substance, which they named Gelfoam (R), to be used as a thrombin carrier for hemostatic purposes. By this method the gelatine is dissolved and a hardening agent added. After the introduction of air bubbles, the mixture is air dried in a metal form and sterilized before use. The product is white, light, tough and porous and can be molded and cut as desired. A slice can absorb 50 times its own weight in water.

Also, for Gladstone's purposes (the examination of cells retained in the sponge), the gelatine sponge appeared, for various reasons, especially suited. Before this, Gladstone had experimented with sea sponges, different types of synthetic rubber and gauze bandages. The gelatine sponge (Gelfoam) proved itself better because of its good absorbence, its ability to be sterilized, simplicity of manufacture and the ability of being worked into all desired forms and sizes. It contained the various chemicals used for tissue fixation and was, in chemical properties, quite similar to human tissue and after paraffin embedding allowed itself to be cut well with a microtome. Loose tissue particles, single cells and liquids are absorbed by the surface and pores of the sponge. By probing the affected cells become completely detached. The gelatine becomes sticky by dampening and holds the loose tissue particles fast. They appear then in the stained section in the hollows of the sponge network in a coagulum of tissue fluid.

Because the gelatine sponge is, however, relatively delicate and tears apart by vigorous rubbing, Gladstone lately changed over to cellulose sponges (Histomed, Inc., Patterson, New Jersey) in cases where rubbing was necessary. Both sponges have a good absorbency and are easily cut with a microtome. The cellulose sponge does not tear as easily, it retains its form better under dampness and compression, it removes and absorbs tissue particles easier from granulating surfaces but is less sticky than the gelatine sponge. Cellulose, in the form of vegetable connective tissue, can be histologically prepared and handled just like an excised tissue preparation. Since both sponges have their advantages, Gladstone has recently used 3 to 4 small pieces of each for a single test. Gelfoam No. 12 is sold in single pieces, sized 2 x 2 x 0.5 cm in sterile packages or in larger blocks sized 12.5 x 8 x 1 cm. They can be cut with a scalpel into strips 8-10 cm. by 1 cm. broad, then into slices from 0.1-0.2 cm thick, giving rise to sponge particles sized 1 x 1 x 0.2 cm.

## II. Technique

The simplicity of the method does not require any special training or experience of the physicians.

The sponge is generally held at the edge with an anatomical clamp. The rectoscope and especially the bronchoscope require especially long and slender sponge-holders. Recently Gladstone worked with a special plier (1923). On the end of an arm of this instrument is found a Rhomboid surface, which serves as a support, making it possible to apply some pressure by rubbing. The other arm is shorter and rough on its outer surface making it possible to scrape hard at cornified bases. The loosened cells and tissue pieces become immediately absorbed by the sponge.

The success of the method depends upon transmission of the cellular material from the carcinoma-suspicious point to the sponge. This point must be seen clearly. This is easily done on the portio with help of a speculum. It can, however, be difficult when the point lies at the end of the broncho-rectoscope. Here it is necessary to have good lighting and good intestinal evacuation (cleaning), eventually by enema and wiping with gauze.

Gladstone uses 2 to 3 pieces of sponge on the portio. The first is rubbed over the entire surface of the cervical lip and the external os. The second piece is split diagonally and inserted into the cervical canal 0.5 to 1 cm

with a long slender clamp. By rotation of the sponge holder in its longitudinal axis the sponge rubs the surface of the mucous membrane. If an ulcer is visible on the portio then a third sponge is rubbed over the ulcer and its edges.

In various articles in 1949 (6,7), Gladstone recommends a wiping of the ulcerated points with dry sterile gauze before pressing and rubbing with the sponge. When a gray-white necrotic covering is present, which he found usually in chronic ulceration, it must be first removed before one can reach the actual ulcer.

In a later article (13) the author noted that a damp sponge on a desquamating surface is ideal for the method. The mixture of expressed secretions and rejected tissue particles is easily absorbed in the sponge pores. A quick dipping of the material into formalin serves to coagulate it and fix it to the surface and on the pores of the sponge. The protein content of the liquid raises its coagulability. Mucus, or also blood in small quantities, may have the same effect. They in no way disturb the test. Gladstone advises, therefore, on damp and desquamating ulcers, not to wipe before sponge application, the wiping being limited to cases with dried scabs and necrosis.

The dry gelatine sponge is pressed against the suspicious area for 30 seconds or wiped over it. Thereby the sponge becomes damp and sticky. Then with a bit of pressure it is rubbed over the ulcer surface, especially along the edges. The author recommends in case of oral carcinoma that a somewhat stable cellulose sponge should be circularly rubbed around the ulcer-base and edges 5 to 6 times. If the sponge is saturated with enough secretions and floating contents this is then recognizable in the staining (coloring) of the white sponge. When no color change takes place, then it must be rubbed more intensely. Later the sponge must be so placed, after embedding, that the used sponge side becomes the fore-surface of the block. Gladstone notes the advantage of this method is that after formalin fixation the sponge can be handled as routine laboratory work. During the paraffin embedding the sponge should be cautiously pressed to eliminate all air bubbles allowing for adequate saturation. The microtome section should be approximately 7-8 $\mu$ thick and can be stained with Hemotoxylin-Eosin.

## III. Diagnostic results for carcinoma of the cervix

The first article of Gladstone (Dec. 1948) on the sponge-biopsy concerned ectocervical carcinoma. Further publications about this topic come from Rich, Carpenter, Angrist, Pollak and Hirst. Gladstone's experience with carcinoma of the rectum, stomach, bronchus, oral cavity and skin were published in 1949-1951.

Microscopically the sponge appears as a network of homogenous material, staining blue with hematoxylin.

Squamous epithelium as single cells, cell groups and tissue particles along with varying quantities of secretions containing polymorphonucleated leukocytes and sometimes numerous Döderlein bacilli are found in the "sponging" (sponge finding) of a normal portio. Sponge findings from the external os often contain sheets of columnar cells and also small quantities of blood which are indicative of abnormal hyperemia or slight wounding of the cervical tissues.

In chronic cervicitis the sponge contains a quantity of purulent exudate. Many of the squamous cells shade over into the cuboidal type with large nuclei, as found in the mucosa with incomplete differentiation.

In typical ulcerating ectocervical carcinoma the sponge contains necrotic, inflammatory, hemorrhagic and neoplastic tissue. Carcinoma cells are present singly, in groups and in tissue particles. They are large and irregular. Their nuclei are deeply stained, showing marked variations in size, form and mitosis.

An adenocarcinoma of the cervical canal can be diagnosed before appearance of clinical symptoms if the characteristic atypical glandular tissues (atypical cuboi and columnar) are present in the sponge findings.

The sponge biopsy discovers symptomless surface ectocervical carcinomas in the characteristic changes of the epithelial cells. Inflammatory, hemorrhagic and necrotic tissue is not yet there.

Gladstone, Angrist and Pollak reported on over 38 cases of ectocervical carcinoma which were first diagnosed through sponge biopsy and then checked by histological examination. Included were nine early, completel symptom-free cases, and 27 advanced cases with bleeding, etc., 35 cases were squamous carcinoma (34 ectocervical, one vulva) and three cases were adenocarcinoma (two cervical canal, one fundus).

Gladstone reported in 1949 (7) on 280 sponge biopsies by means of which two early and four advanced ectocervical carcinomas were discovered.

Angrist, Rich and Carpenter limited themselves to examination of suspect cases. From 68 suspect cases, 17 were positive.

Hirst's comparison of the results of Papanicola smears and sponge biopsies of ectocervical carcinoma during pregnancy demonstrates that the smears give mo false positive results and sponges more false negative r sults.

In March, 1951, Gladstone reported the biopsy r sults of 641 female patients (242 in-patients, 256 out-patients, 143 private cases). The microscopic results of sponge biopsy in 189 cases checked exactly with histolog ical preparations.

All in all, there were 16 cases of cervical carci noma. In nine cases there was, clinically, suspicion of ectocervical carcinoma evidenced by vaginal bleeding, visible ulceration of the ectocervix or induration of the cervix and surrounding tissue. The other seven cases were clinically without symptoms. Among these seven were two cases of adenocarcinoma of the cervical canal and five cases of early superficial squamous epithelial carcinoma of the ectocervix.

## IV. Views of the American authors concerning the adva tages and disadvantages of the sponge biopsy

Gladstone reports in his own articles about car cinoma cases which were discovered through sponge biopsy and verified by probe excision.

Because of the type of patient selection and num ber that were examined with the sponge method it is not feasible to numerically compare this method with others There are, however, definite advantages and disadvantages when compared with surgical biopsy.

### A. Advantages

1. The method causes no trauma.
2. The material can be taken by non-specialized personnel.
3. The simplicity of the method allows for a greater reapplication on the same patient.
4. The method can be used routinely in every gynecological examination.
5. Papanicolaou smear necessitates fixation and staining procedures which are not used in the average laboratory. The sponge can be handled with the ordinary histological procedure.
6. The cellular configuration corresponds to that of the usual histological preparation; the pathologist is accustomed to this pictu
7. A complete smear need not be examined, f the questionable cell-groups are found on the edge of the sponge or in superficial lay ers. This means a great saving of time.

B. Disadvantages

1. The advantages of selective election of material from the cervix are the same as in the direct smear taken with the Ayre spatula or scraping with a sharp spoon. After this the material can also be handled histologically by embedding in paraffin or can be handled cytologically after fixation and staining (Papanicolaou's original method).
2. Preparation of a sample with Papanicolaou method takes only 30 minutes. Handling the sponge histologically takes at least 10-12 hours.
3. The cell configuration corresponds to the usual histological preparation, however, the main advantage, namely the over-all view of the histological structure is often missed. Histological preparation of scraping or selective biopsy allows one to judge the presence of carcinomatous invasion.

   In our experience the sponge method makes a tissue diagnosis in the true histological sense often impossible. The diagnosis is far better supported by cytological properties in view of the single cells or cell groups. Therefore, the diagnosis means a transposition for the histopathologist and belongs more to the field of the cytologist.
4. The examination of a sponge preparation takes practically as long as a smear. It at least takes longer than a histological preparation, since one cannot orientate one's self in connection with tissue structures in most cases.
5. One of the most important advantages of cytology, namely the survey over a great area by spreading the cells on a glass slide, is lost.

We feel that sponge biopsy, in most cases, does not give the definite diagnosis as is given by true biopsy. In a suspicious sponge preparation, biopsy by selective biopsy or conization has to be followed. Sponge biopsy can exclude harmless cases as well as normal cytology can do. Therefore, we feel that the sponge-method is by far too time consuming and demands as many technical steps as a histological preparation without being as conclusive as a normal biopsy. We would always prefer cytology in its classical sense for screening procedures and go on to do true biopsies in suspicious cases.

Bibliography


1. Angrist, A., Rich, J. and Carpenter, F.: Am. J. Ob. Gyn. 59:1029, 1950.
2. Angrist, A. and Pollak, A.: Am. J. Publ. Health 40: 828, 1950.
3. Ayre, J. E.: Am. J. Ob. Gyn. 53:609, 1947.
4. McCreary: Arch. Dermatol. & Syph. 62:561, 1950.
5. Gladstone, S. A.: Am. J. Medic. 5:849, 1948.
6. Gladstone, S. A.: Am. J. Clin. Pathol. 19:92, 1949.
7. Gladstone, S. A.: New England J. Med. 241:48, 1949.
8. Gladstone, S. A.: Cancer 2:604, 1949.
9. Gladstone, S. A.: Am. J. Clin. Path. 19:891, 1949.
10. Gladstone, S. A.: New York State Med. J. 49:2687, 1949.
11. Gladstone, S. A.: Arch. Dermatol. 62:380, 1950.
12. Gladstone, S. A.: Am. J. Surgery 81:307, 1951.
13. Gladstone, S. A.: Am. J. Surgery 9:104, 1951.
14. Gladstone, S. A.: J. A. M. A. 145:1238, 1951.
15. Hirst, J. C.: Am. J. Ob. Gyn. 61:860, 1951.
16. Stoll, P. and Zeitz, H.: Zentralblatt Gyn. 74:769, 1952.


## DISCUSSION

WOLFGANG KORTE, Bonn, Germany:

Every method of examination which pays attention to the physiological conditions of the utero-vaginal canal can, on principle, be highly valued. It is another question, though, as to how the practical use will be effected.

Gladstone has created and prefected his sponge technique with remarkable aptitude. I presume that this idea came to him more from cytology than from histology. I think that the method, which opens a third dimension to cytology, is extraordinarily useful for function tests and for cytochemical explorations. A smear can be stained only once. The paraffin embedded sponge supplies series of sections of exfoliated material from a fixed place at a certain moment.

As to the practical clinical use I agree with Stoll. For routine examinations the method does not yield more information than the smear. It can hardly be compared to histological preparations, because every real tissue relation is lacking. Expense of time and labor is too great with respect to its use for only cytological diagnostic results.

## CLOSING REMARKS

PETER STOLL:

I cannot accept Korte's idea that sponge biopsy exhibits advantages for cytochemical explorations. The application of cytochemical methods to paraffin-embedded material is limited; I would always prefer doing multiple smears and varying the method of fixation for further cytochemical procedures. Sponge technique has its main advantages in other fields of cytology (stomach, rectum, etc.), rather than in gynecology, where collecting material by purely cytological methods or by biopsy is easy.

# IF ONE PREPARES MORE THAN ONE SMEAR PER PATIENT, COULD ONE PREPARE THESE SMEARS ON ONE GLASS SLIDE OR DOES ONE HAVE TO PUT THEM ON SEPARATE GLASS SLIDES?

GUNTER F. BAHR AND
GEORGE L. WIED
Washington, D.C., and
Chicago, Illinois, U.S.A.

In a previous paper (1) we have had the opportunity to show that the above question may be answered in the affirmative. There is no question but that one should prepare more than one smear per patient in order to guarantee proper evaluation of the smear (2,3,4,5,6). For example, (a) cytohormonal evaluations should not be made from cervical scrapings, and microbiological classifications of the vaginal flora cannot be made from endocervical smears; (b) carcinomas in situ and early invasive carcinomas are usually most accurately and readily detected in cervical and endocervical smears; (c) cervical scrapings, on the other hand, are not useful for the detection of endometrial carcinoma; and finally (d) three smears must be prepared in order to determine in which part of the genital tract any present inflammatory reaction is localized. Thus it is important that vaginal, cervical and endocervical smears are available in every case for comparative evaluation, if one does not wish to intentionally decrease the scope of cytology and to intentionally lower the diagnostic accuracy of exfoliative cytology.

During the past five years these three cytological smears have been prepared in the Chicago Lying-in Hospital on an individual glass slide. The objections which are advanced by the casual visitor to the laboratory, are usually that (a) the smears will dry before they are fixed, (b) one does not have enough material for examination because there is not enough space to spread it, and (c) that the smears would be mixed on the slide and/or the order of their application reversed in some cases. These objections are generally raised by individuals who prepare only one slide in their own institution as a routine technique, and who have never performed a comparative study on the accuracy of the three-smears-on-one-slide technique as compared with the one smear technique, and/or as compared with the three-smears-on-three-slides technique.

From the above paper (1) we would like to show a few tables to demonstrate our findings.

Table I shows the diagnostic accuracy of exfoliative cytology as determined in a comparative study on 100 patients with cervical carcinoma: one smear each was prepared from the ectocervix and spread on an individual glass slide, whereas on the other glass slide there were vaginal, cervical and endocervical smears:

Table II shows that the diagnostic accuracy of the "Three-smears-on-one-slide" and the "Three-smears-on-three-slides" techniques are practically identical, as demonstrated on 1000 patients in a comparative study.

Table III demonstrates the results obtained on comparative studies on 100 patients with cervical and en-

Table I
(Papanicolaou Classification)

| | Cervical smear only | Three-smears-on-one-slide |
|---|---|---|
| Class 1 | 3 | 0 |
| Class 2 | 6 | 1 |
| Class 3 | 17 | 16 |
| Class 4 & 5 | 74 | 83 |
| Total Error | 8% | 1% |

Table II

Specimens of 1000 Clinic Patients Prepared According to Both Techniques Evaluated in the Five Classes of Papanicolaou

| | "Three-smears-on-one-slide" technique (vag., cerv., endo.) | "Three-smears-on-three-slides" technique (vag., cerv., endo.) |
|---|---|---|
| Class 1 | 72.6% | 72.2% |
| Class 2 | 26.5% | 26.9% |
| Class 3 (suspicious) | 0.5% | 0.5% |
| Class 4 & 5 | 0.4% | 0.4% |

Table III

Specimens of 100 Patients with Cervical and Endometrial Carcinoma Evaluated According to the Five Classes of Papanicolaou

| | "Three-smears-on-one-slide" technique | | "Three-smears-on-three-slides" technique | |
|---|---|---|---|---|
| | Cervical Carcinoma | Endometrial Carcinoma | Cervical Carcinoma | Endometrial Carcinoma |
| Total | 80 | 20 | 80 | 20 |
| Class 1 | 0 | 4 | 0 | 4 |
| Class 2 | 1 | 4 | 1 | 3 |
| Class 3 (suspicious) | 15 | 6 | 14 | 7 |
| Class 4 & 5 | 64 | 6 | 65 | 6 |
| Total error (no. of cases) | 1 | 8 | 1 | 7 |

dometrial carcinoma examined with smears prepared according to the "Three-smears-on-one slide" and the "Three-smears-on-three-slides" techniques.

From these two Tables it is evident that the diagnostic accuracy is practically identical whether one employs this or that preparation technique, as long as three smears are prepared at all.

The argument that smears dry prior to fixation may be valid if the person who prepared the smears spreads each smear on the glass slide after it is prepared. This is, however, not the way it is done in our clinics: the clinicians are advised to take the smears and to keep the material on the respective applicators until all three smears are prepared. Only then are the three specimens spread on the glass slide and immediately put into the fixative.

For more detailed information we would like to refer to the paper mentioned above (1).

Summarizing, we would like to say that (a) the "Three-smears-on-one-slide" technique is definitely superior to the technique of preparing only one smear per case, and that (b) the "Three-smears-on-one-slide" technique is practically as efficient and (c) far less expensive (as far as expenses for glass, staining material, storage space, and staff time are concerned) than the "Three-smears-on-three-slides" technique.

Bibliography


1. Wied, G. L. and Bahr, G. F.: Obstetrics and Gynecology, 14:362, 1959.
2. Boschann, H.-W.: ACTA, Union against Cancer 14: 372, 1958.
3. Pundel, J. P.: Acquisitions Recentes en Cytologie Vaginale Hormonale, Paris, 1957, Masson.
4. Simon, T. R., Durfee, G. R. and Ricci, A.: Transactions, Third Annual Meeting, Inter-Society Council, 1955.
5. Wied, G. L.: Am. J. Clin. Path. 25:742, 1955.
6. Symposium on Techniques for Endometrial Cytological examinations: Acta Cytologica 2:566, 1958.



JOSEPH S. SONG

Providence, Rhode Island, U.S.A.


For the cytological cancer screening survey we do not prepare multiple type of smears on each patient. However, we do recommend multiple smears be taken on selected cases. In our tumor clinic, we usually take several of the endocervical os aspiration smears applied by specially designed pipettes, cervical scraping smears using the wooden spatula, and routine vaginal aspiration smears made on separate glass slides.

By examining multiple smears, we find that the accuracy for the screening survey can be increased, and satisfactory evaluation of biological data be obtained. We have been asked to use the single smear method on gynecological patients utilizing the single glass slide on which three different segments are made. We found that a restricted field for different types of smears made on one glass slide was too small to spread the material without intermixture. We believe that different types of genecological smears should be made on separate glass slides for a more accurate interpretation.

## DISCUSSION

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany:

If the authors obtained equally good results with

both methods in their series of tests, this should be reason enough for similar comparative examinations in other hospitals. There is no doubt that the diagnostic safety of cytology is elevated by taking the smears from different spots at the same time.

MARCEL GAUDEFROY, Lille, Nord, France:

Like Song, I was afraid that the diagnostic accuracy of the "three-smears-on-one slide" might be lower than that of the "three-smears-on-three-slides." After having read the statements of Bahr and Wied, I decided to use the described method and I was surprised to observe approximately the same results as these authors. I quite agree that the technique of "three-smears-on-one-slide" is very reliable: to use it or not is a matter of habit. But I would like to say to Wied that the technique of "three-smears-on-one-slide" is not definitely superior but only equal to the "three-smears-on-three-slides" method. In addition, having bought some special VCE slides, I found that these excellent slides were much more expensive than ordinary slides.

EMERICH von HAAM, Columbus, Ohio, U. S. A.:

While I am inclined to agree with Song's opinion, I became convinced by the demonstration of Wied and Bahr that the VCE one-smear-technique is as accurate as the VCE three-smear-technique. However, in order to make it so, more skill and care are necessary in the preparation of the smear to obtain a sufficient number of cells and an accurate separation of the smear types.

WOLFGANG KORTE, Bonn, Germany:

If one prepares more than one smear per patient, could one prepare these smears on one glass slide or does one have to put them on separate glass slides?

The three-smears-on-one-slide technique, reported by Wied and now again put to discussion by Wied and Bahr, certainly has advantages. However, it requires a careful "education" of those who take the smears. The three-smears-on-one-slide are simultaneously fixed and then stained. This eliminates one source of error—the mixing up of the various slides. In my opinion the comparable diagnostic accuracy on using the three-smears-method is unquestionable. The careful gynecologist will be able to prepare three smears on one slide. The objection of Song is valid, if the smears from the three regions are not uniformly prepared.

HANNES KREMER, Vienna, Austria:

From our experience, based on over 70,000 smears, we believe that smears taken from different areas of the female genital tract and put on only one slide is sufficient for the diagnosis of a carcinoma of the cervix, as well as for the functional diagnosis. At the First University Clinic for Obstetrics and Gynecology in Vienna, we have maintained the following practice:

Close to the engraved name of the patient, the smear of the visible lesion (for example, erosion of the cervix) is placed. Next to that is the smear which has been taken from the cervical canal, and next to this the smear from the posterior fornix or from the lateral vaginal wall.

LUIS MONTALVO RUIZ, Madrid, Spain:

I agree with Song that, in order to make a complete study of hormones, malignancy, and vaginal flora, it is necessary to prepare three smears on separate slides. Thus, we obtain more surface area and more material for study in each smear. When taking three smears on one slide, the first smear will tend to dry, whereas the reverse is true when using three separate slides. Nevertheless in cancer screening in a large screening project, the three smears on a single slide are less expensive; this is the advantage we found in this procedure. In the Cytology Department of Cancer Prevention, we make the three smears on a single slide, following Wied's advice in his speed at the Instituto Provincial de Obstetricia de Madrid in 1956. From 1954 to then we had made smears on three separate slides. This is still the method of choice in our private practice for the above mentioned reasons.

WOLFGANG WALZ, Heidenheim, Brenz, Germany:

The exact studies of Wied and Bahr have impressed me very much. They prove that three smears on one slide suffice. Here in Germany cytology is only a young science and still in the process of propagation. In many places the installation of a cytological laboratory depends very much on financial and personnel questions. Therefore, it is of great importance that by the described method the cost can be reduced without loss of accuracy and efficiency.

We ourselves only take two smears: one from the cervix and one from the endocervix, while we usually omit the vaginal smear. However, each patient undergoes a colposcopical examination which may be regarded as a substitute for the vaginal smear, with the limitations of the method always in mind. In the case of conspicuous colposcopical findings we take a guided smear from the vaginal wall. I believe that by the combination of the two methods in the ascribed way, the efficiency and economy may be increased.

May I draw the conclusion that the three-smears-on-one-slide method should be employed at places where cancer detection is done by cytology alone, while, with the additional application of colposcopy, two smears, one from the cervix and one from the endocervix, suffice? It is obvious that for the hormonal evaluation, the vaginal smear is indispensable.

## CLOSING REMARKS

GEORGE L. WIED:

May I thank the discussants for their contributions which vividly show the interest in this technical question, and may I answer as follows:

To Bickenbach and Soost: it would certainly be very good if other institutions could perform similar comparative examinations. It would be logical, however, that the more material one has the higher the diagnostic accuracy will eventually be. If, from the examination of the first smears, there is the slightest suspicion in our mind that malignancy could be present, we liberally request repeat smears and thus "increase the surface of the specimens." I have yet to see one hundred percent normal Three-smears-on-one slide specimen which was prepared from a malignant epithelial lesion. There are always some abnormalities present.

To Gaudefroy: I was glad to see that this discussant went to the trouble of practically examining the Three smears-on one-slide technique. Gaudefroy, like us, found after this study that the diagnostic accuracy was approximately the same whether he used the Three-smears-on-one-slide method or the Three-smears-on-three-slides method. We are, however, slightly misquoted by Gaudefroy when he says that in the paper we stated that "the Three-smears-on-one-slide technique is definitely superior to the Three-smears-on-three-slides technique." What we said, instead, is that the Three-smears-on-one-slide method is definitely superior to the One-smear-on-one-slide technique (namely the usually obtained cervical smear only). Another objection raised by Gaudefroy, is the price of the VCE-slides, as com-

pared with slides which he bought in Czechoslovakia. This objection also needs some clarification. The fact that a manufacturer has commercially begun producing slides with a frosted "V," "C," and "E" and frosted partitions of the glass slide has nothing to do with the practice of this type of smear preparation. Through many years in my former laboratory in Europe we prepared the three smears on one slide (and they are still doing it there) on regular glass slides without any frosted letters or partitions on it. We had those frosted ones made only because sometimes we received the vaginal smear on the part of the slide where we expected the endocervical smear to appear. In order to eliminate the slightly time-consuming task of identifying from the smears endocervical mucus and cellular constituents, we use these frosted slides, but they are by no means a conditio sine qua non. Another point in this connection: I have been informed that another manufacturer now produces these slides for a considerably lower price.

To von Haam: Again there is also no doubt in my mind that the Three-smears-on-three-slides technique offers the larger area for examination than the Three-smears-on-one-slide method. I do not quite see, however, where skill would play a significant role. For the One-smear-on-one-slide one moves the wooden spatula lengthwise across the glass slide. When preparing the smear for the Three-smears-on-one-slide technique, one simply changes the direction of the movement of the spatula. The space for each smear which is circumscribed with the frosted subpartitions is just sufficient for the width of the wooden spatula. The question is: Because of the larger area present for examination, will cancer cells be present in a smear which has been smeared out lengthwise when they are not present in a smear which is performed in the other way? We do not think so because it does not seem quite consistent with experience that in an otherwise normal smear there should be only a single area of malignant cells which was not spread due to lack of space.

To Korte: The discussant states that careful education of the individuals preparing the smears will be required for the Three-smears-on-one-slide technique. I agree that some information must be given to the clinician, but this is usually rather simple: we just advise them NOT to spread the specimens on the glass slides before all three specimens have been taken. The material remains as a "thick drop" on the two wooden spatulas (of vaginal and cervical smears) and the nurse holds these two wooden applicators until the clinician has prepared the endocervical smear, which he spreads immediately on the glass slide. Then he takes the other two wooden tongue blades and spreads the material from these on the glass slides. I have never seen even the slowest intern or resident succeed in being so slow that the thick drops of vaginal and cervical mucus dried on the wooden applicators before he made his endocervical specimen. Of course, it would be wrong to immediately smear the specimens when they are individually prepared. In this case at least one smear could possibly air dry before the third smear is spread.

To Kremer: This discussant uses practically the same technique as we do; namely a vaginal, cervical, and endocervical smear on one glass slide. He states that he found this technique satisfactory for cancer detection in his material of over 70,000 cases.

To Montalvo: If one prepares the smears as described above one will not find that air drying is significant. If Montalvo spreads his smears, however, after each preparation of the specimen, he simply uses a wrong technique. There is another point I would like to mention from the discussion of Montalvo: He states that hormonal and microbiological readings would be more accurate if prepared on glass areas larger than those available on the VCE slide. I cannot subscribe to this statement, since just these two evaluations require the smallest slide space of all cytological evaluations. We have never found that we had to examine more than ten to twenty square millimeters of a representative specimen to give any of those two readings.

To Walz: This discussant points out that the cervical and endocervical smears are adequate for cancer screening, but that vaginal smears are requested for hormonal evaluation. Walz points out the main problem as evidenced in the discussions, namely that some of us preselect our cases (a) for cancer screening and (b) for "other diagnostic purposes." Just as a clinician never knows which of his patients could have a subclinical early carcinoma, he could not know, for example, which of his patients has a subclinical trichomonas infestation, bacterial cytolysis, absolute hyperfolliculinia, etc. As it does not seem quite logical to preselect patients for cancer screening, it seems even more illogical to preselect gynecological patients for evaluation of their non-malignant disturbances, such as infestations, infections, hormonal disturbances and imbalances. After all, the vast majority of all our gynecological patients do not come to us only because they wish to be screened for cancer. Considerably more often they come to us because they are concerned about discharge, amenorrhea, menstrual disorders, pruritus, subfertility, pregnancy and other reasons which are clearly not clinically primarily suspect for a malignant lesion. In all those cases the cytological smear may be of some diagnostic or prognostic aid. Another point is that one can go back to the old smears of the patient and can correlate the previous cytohormonal and microbiological findings with the present ones, if one routinely prepares three smears. Scientific studies can be done considerably more easily if one has the same technique of smear preparation for all cases, and not just for some preselected individuals.

Before we get into another heated discussion about some minor problems, I would like to state that we have found this type of smear preparation satisfactory on material of more than 100,000 cases, and that we feel that it is superior to the usually obtained single cervical smear technique. The gain is, however, saving of glass material, saving of time, satisfactory diagnostic accuracy, and usefulness for all gynecological patients and not only for those who fall into a preselected group of "cancer survey projects."

# IN CASE OF A SUSPICIOUS ECTOCERVIX, SHOULD A BIOPSY BE DONE WITHOUT CYTOLOGY?

FREDRICH BAJARDI
Graz, Austria

If it would be possible in every case of a suspicious cervix to perform a cone biopsy with subsequent preparation of serial sections, a missed carcinoma, due to insufficient histology, could be almost certainly eliminated. In this case an additional cytological evaluation would be superfluous.

If, however, one has to depend upon a small biopsy, as is often the case, the great danger of "biopsy on the wrong site" should be born in mind. A check on our own material revealed that with smaller biopsies (punch biopsy, Schiller scraping) only 258 (85.4%) out of 302 preclinical carcinomas could be diagnosed primarily by histology. Thirty-two of the remaining cases have been verified with the second biopsy (10.6%) and only 11 cases (3.6%) with the third biopsy. In one case the carcinoma could be detected only with the fourth biopsy. It must be emphasized that all these biopsies were guided with the aid of the colposcope. Thus, even under these optimal conditions, 44 carcinomas would have been missed with the first histological examination (14.6%). This error undoubtedly would even be larger if one would take biopsies only from suspicious looking sites, i.e., without the help of the colposcope.

Therefore, we believe that cytology should be done in every case, since it is able to control the histology. In those cases where there is positive cytology with negative histology there should always be a second biopsy performed. Should one eliminate cytology a great percentage of the histological errors would remain undiscovered.

On the other hand, performing a biopsy should not depend upon the result of the cytological examination alone, due to the possible error of the cytological evaluation. Thus, in this case histology has to control the possibly negative cytology. According to our experience, however, it is much safer to use colposcopy routinely instead of inspecting the cervix with the naked eye and from this determine whether or not one should do a histological examination. With this combined method it was possible to detect 304 out of a total of 306 preclinical carcinomas (98.7%) (1).

MARIA KAWECKA, JADWIGA BYLINA,
IRENA KLINIAK,
AND HANNA STARKIEWICH
Gliwice, Poland

We are presenting the experience of the Oncological Out-Patient Department at Gliwice, Poland, and therewith trying to answer the question, whether or not, in a case of a suspicious ectocervix, biopsy should be done without cytology?

Twenty thousand patients with erosion have been examined in the period from 1952 through 1957.

As a rule two separate smears have been taken from every patient, both from the ecto- and endocervix. The Papanicolaou technique was used. Biopsy has been done, whenever a Papanicolaou Class III, IV and V was established.

Thus, 561 cases of cancer and so-called borderline cases have been detected and histopathologically proved among 20,000 women with erosion (Table I). We considered as borderline cases: (1) atypia majoris gradus suspecta quoad carcinoma in situ, (2) casus limitans.

The cytological examination revealed Papanicolaou Class III, IV, V in only 551 patients. The 10 remaining patients belong to the false-negative group, inasmuch as they have been diagnosed as Class II. The erro-

Table I

Cases of Carcinoma Detected among 20,000 Patients with Erosion

| | |
|---|---|
| Carcinoma in situ et casus limitans | 359 |
| Microcarcinoma invasivum | 99 |
| Carcinoma invasivum | 93 |
| Adenocarcinoma | 10 |
| Total | 561 |

neous diagnosis represents, thus, 1.78%. The erroneous diagnosis occurred mainly in the initial period of our work and was due to the lack of experience of the cytologist. We stated after re-examination of those smears, that the cancer cells had been overlooked, mainly whenever there was an abundance of blood and a scarcity of cellular elements in the smear. Such a picture is prevalent in cases of invasive cancer. On the other hand, the early and especially the carcinoma in situ produces a good smear, as the desquamation of cells in such cases is rich, and bleeding caused by the cytological procedure scarce.

ages of accuracy can be considered as equal for both diagnostic procedures. By means of using both methods simultaneously, it has been made possible to further detect 11 cases of cancer. Apparently, the applying of both methods is the most efficient procedure. However, this is impractical since at least four specimens should be taken for biopsy from each ectocervix.

The appearance of the ectocervix is misleading (Table III). The gynecologist considered 1,137 cases of our material as requiring biopsy. Moreover, he labelled 526 of them as definitely suspicious. In 1,137 cases both cytology and biopsy were done, however, cancer has been found by means of biopsy only in 121 cases or 10%. Thus, biopsy was unnecessarily done in 90% of these patients.

Table II

The Cytological and Histopathological Diagnosis in 561 Carcinoma Cases

| First Cytological Diagnosis | | First Biopsy | |
|---|---|---|---|
| Class III, IV, V | 551 | Carcinoma | 519 |
| Class II (fasle negative diagnosis) | 10 = 1.78% | Atypia majoris gradus | 28 |
| | | Erosio (diagnosis did not conform with definitive biopsy) | 14 = 2.49% |

The opposite can be said as to the relationship between the accuracy of the histopathological examination and the invasiveness of the carcinoma (Table II). The pathologist failed to diagnose cancer with his first examination in 14 patients among 561 definitely confirmed cases and in 28 further cases of this group only a diagnosis of "atypical epithelium" was established. It was not until the re-examination of the biopsy specimen was done, that the cytological diagnosis of cancer was confirmed.

The re-examination was performed after and because of the cytological diagnosis, Class V. Thus, the first pathological examination proved to be erroneous in 14 cases, or 2.49%.

Four hundred and twenty-nine cancer cases of the 561 histologically proven ones presented no macroscopic evidence of cancer and could not be detected by other than cytologic studies. Both biopsy and cytology were done in 132 cases, whereby the cytology turned out to have detected 123 cases of cancer, while the pathological examination revealed 121 cases of cancer. But the cases were not completely identical in these two groups (Table III).

The results are identical in 111 cases.

Cytology failed to detect cancer in ten cases where the biopsy established the diagnosis. On the other hand, the pathologist failed to diagnose cancer in 11 cases diagnosed by means of cytology. Thus, the percent-

We are, therefore, of the opinion that prior to biopsy, cytology should be performed. However, control examination of patients, diagnosed, as Papanicolaou Class II, and treated, should be performed every six months. This could reduce the percentage of false-negative results of cytology.


## HANNES KREMER

Vienna, Austria


As a result of examinations at the First University Clinic for Obstetrics and Gynecology, Vienna, Austria (1), a series of 130 carcinomas of the uterine cervix revealed the unreliability of the macroscopic diagnosis of pathologic changes on the cervix. Thirty-five cases (27 carcinomas in situ, 8 carcinomas of the cervix, Stage I) did not seem to be suspicious when examined by highly experienced gynecologists. The other 95 cases (59 carcinomas of the cervix of the uterus, Stage I, 36 carcinomas in situ) gave reason for suspicion of the malignant change when macroscopically examined, i.e., by

Table III

The Comparison of the Results of Simultaneously Performed Biopsy and Cytological Examination in 1,137 Cases of a Suspicious Ectocervix

| Results Conform | | Results do not Conform (in Favor of Cytology) | | Results do not Conform (in Favor of Biopsy) | |
|---|---|---|---|---|---|
| First cytology positive<br>First biopsy positive | 111 | First cytology positive<br>First biopsy negative | 11 | First cytology negative<br>First biopsy positive | 10 |
| First cytology negative<br>First biopsy negative | 1005 | | | | |
| Total | 1116 | Did not conform | 11 | Did not conform | 10 |

speculum. Now the question arises: could a final diagnosis have been achieved by a biopsy, if it were performed on several areas of the cervix? Certainly not in all cases. For it has been rather often proved histologically, in serial sections of the cervix, that carcinomatous parts were located only in circumscribed areas. Biopsy alone could not have possibly revealed all of these carcinomatous parts.

Another series of examinations of 115 cases of carcinomas in situ (2) proves that only 64 cases seemed to be macroscopically suspicious for malignancy. But also in these cases a biopsy performed on several parts of the cervix, could not have been successful in every case. It could be proved by histology in serial sections of the amputated cervix that the carcinoma was developed only in circumscribed areas of the surface of the cervix, and in some cases only inside the cervical canal. Those carcinomas in situ which were situated only inside of the cervical canal would probably not have been detected by biopsy. Generally, the percentage of endocervical carcinomas in situ is given at a range of from 2-30%. In our material seven cases of endocervical carcinoma in situ were noticed (6%).

By cytology, however, cells of the whole surface of the cervix and from the cervical canal are seen and examined. In case of a positive cytologic report, biopsy alone will not be sufficient to reach a final diagnosis. It would be better to perform a conization or amputation of the cervix, followed by a fractioned D & C of the rest of the cervical canal and of the cavity of the uterus. At least two negative cytologic reports will, in spite of a macroscopic suspicion, be reason enough not to do any further diagnostic procedures, if, as is the general practice at the First University Clinic, the negative cytological report is confirmed by other routine cancer detection methods (colposcopy, and colpomicroscopy).

Biopsy, therefore, seems to be an insufficient diagnostic procedure in the case of a suspicious erosion. But here also an exception is to be found. This is the macroscopically obvious carcinoma of the cervix (for example, exophytic growth). It is not necessary, most of the time, to take a smear for cytological examinations in these cases. Instead we need the histological report of invasive carcinoma in order to perform radical treatment.

Bibliography


1. Janisch, H., Klein, R. and Kremer, H.: Geburtshilfe-Frauenheilkunde 19:63, 1959.
2. Kofler, E. and Kremer, H.: In print.


## JOSEPH S. SONG.

### Providence, Rhode Island, U.S.A.

Most clinicians seem anxious to perform a biopsy on the cervix when there is an area of leukoplakia, a large area of erosion, and abnormal discharge or bleeding from the cervical stump in order to confirm the diagnosis. It is our experience that cytologic findings usually correlate the histological diagnosis when the smears are properly made from the suspicious area of the cervix. A considerable number of cone biopsies were performed from the area of a large erosion simultaneously with the cytologic examination. When the cytologic findings are negative in cases of suspicious ectocervix, the histological diagnosis is usually negative. It is our policy to urge clinicians to take several scraping smears directly from the suspicious lesions on the ectocervix before they consider biopsy procedure.

## PETER STOLL

### Heidelberg, Germany

The line of action for the diagnosis of cervical cancer should be the following:

A. General Procedures
   1. History (discharge, contact-bleeding, irregular bleeding)
   2. Gynecological examination
      Inspection of ectocervix, iodine-test, palpitation after B
B. Special Procedures
   1. Colposcopy (Hinselmann)
   2. Cytology (Papanicolaou)
   3. Histological examination

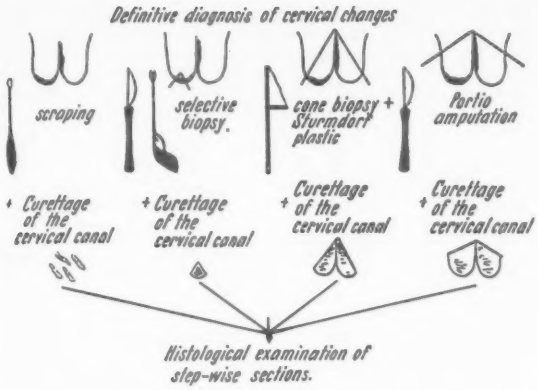
The important question is "when does the suspicion of malignancy arise?" At this very moment every effort should be made to come to a definite diagnosis.

1. If the history is suspicious and simple gynecological examination reveals tissue defects, ulceration or tumor, biopsy is the method of choice and should be done immediately in order to avoid delay of diagnosis. In those cases the possibility that the biopsy fails to catch the lesion is very rare. Nevertheless, cytology should be done in addition to the biopsy, but it is of secondary importance. Everyone knows, that in the case of a full blown ectocervical carcinoma with destruction and inflammation, smears are sometimes doubtful or even negative, because there are only cell detritus, blood and leukocytes on the slides.
2. If the history is suspicious and simple gynecological examination reveals only a red spot ("erythroplakia" after Navratil) around the external os, colposcopy and cytology have to be done. It might be, that under the colposcope, one area of the red spot might seem to be highly suspicious. In such a case I would immediately take a selective biopsy of this region in addition to taking the smears. If the colposcope reveals bleeding from the cervical canal, endocervical smears have to be taken very carefully. If there is no suspicion under the colposcope, direct smears from the ecto- and endocervix are taken.
3. If the history is not suspicious and the simple gynecological examination seems normal, this is the most important field of cytology: early diagnosis.

It might be unnecessary to say that colposcopy and cytology are able to save time and expensive histological examinations, and that colposcopy and cytology should be done routinely in every patient seen in the office. However, when there is already carcinoma on the ectocervix seen with the naked eye and if the experienced doctor already smells cancer, I see no reason to delay biopsy.

In taking biopsies, the more radical methods are better than taking only one small selective biopsy. This is said, not only from the diagnostic, but also from the therapeutic standpoint. If the ectocervix presents symptoms, it should be removed.

I would like to mention a very interesting case of a 39-year old girl (technician in one of our departments) who suffered with an allergic edema during the time of her menstrual period. Otherwise she felt entirely healthy and did not complain of gynecological symptoms,


Definitive diagnosis of cervical changes
scraping
selective biopsy.
cone biopsy + Sturmdorf plastic
Portio amputation
+ Curettage of the cervical canal
+ Curettage of the cervical canal
+ Curettage of the cervical canal
+ Curettage of the cervical canal
Histological examination of step-wise sections.

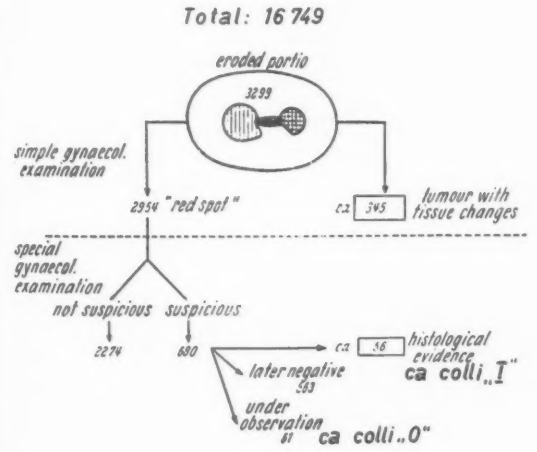
such as discharge, bleeding on touch, etc. On simple gynecological examination an unsuspicious red spot was seen around the cervical os. Biopsy (selective) was done at 12 o'clock only, in order to obtain some material for histochemical research. A smear was taken as well and turned out to be positive. Biopsy revealed, in addition to normal squamous epithelium, an area of metaplastic epithelium without any indication of malignancy.

The patient came back after four weeks for another consultation. Again the smear was positive. On colposcopy the suspect area of the red spot could be defined on the posterior lip of the ectocervix and a fairly wide sharp-knife excision was done at 6 o'clock. On serial sections there was nothing indicating malignancy, the histological diagnosis was metaplastic epithelium with inflammation.

After another four weeks a few smears again were positive. We decided to do a conization which revealed to our great surprise, squamous cell carcinoma infiltrating the whole lateral wall of the ectocervix. The surface of the tumor, which itself was the size of a cherry, was only 0.5 to 0.5 cm in diameter at 9 o'clock.

This case shows, that blind biopsies are of little value and that also the guided biopsy, done under the colposcope may miss the area of malignancy. Therefore, the immediate biopsy should be limited to such cases, in which a tumor is already present to the naked eye on inspection.

In our Out-patient Department we have seen,




during 1951-1954, 16,749 patients coming to us for the first time. In 345 cases, a tumor was already present on inspection and biopsy was done immediately and revealed carcinoma.

In 2,954 cases only a red spot was present at the cervical os. After colposcopy and cytology, 2,274 cases could be excluded from further investigation, 680 cases were suspicious. From these, by further observation, 563 lesions turned out to be benign, 56 were carcinoma by histological evidence, and 61 are still under observation.

Among the remaining 13,450 patients with macroscopically innocent cervices, employment of special diagnostic methods (colposcopy and cytology) led to the discovery of 10 additional cases of invasive cervical carcinoma.

## SUMMARY

If, upon simple inspection, there is found a tumor of the ectocervix, then cancer is more than just a suspicion and immediate biopsy is indicated.

In cases of an eroded cervix, the frequency of cervical carcinoma is 1:53. Therefore, the erosion shows cause for suspicion. But, in these cases, carcinoma can hide itself within the erosion and may not be diagnosed as such by a selective biopsy. When colposcopy and cytology are negative, then it is only necessary to keep the patient under observation. When colposcopy and/or cytology are positive, then it is necessary to perform a cone biopsy.

## DISCUSSION

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany:

We are of the opinion—agreeing with the authors—that before any biopsy or conization is done a cytological smear ought to be taken. The cytological examination is an additional affirmation. We also review suspicious cases. When the first histological examination shows a negative result and only further investigations, inaugurated by positive cytological findings, are able to detect the malignant process.

If the cases are clinically suspicious but histologically benign, the negative cytological findings are an additional guarantee.

MARCEL GAUDEFROY, Lille, Nord, France:

It seems that the question has been perfectly studied. However, I would add the following considerations: If there are false-negative cytological responses, there are also false-negative histological responses. Moreover, it is necessary to be in agreement on the meaning of the word "suspicious." If the cancer is macroscopically evident then a biopsy must be performed immediately. If the cervix is not perfectly normal and sound, it is not necessarily suspicious. In my mind, all the cervices which are not perfectly normal must be studied with the colposcope, and it is the routine of my practice. If the abnormality of the cervix is evidently inflammatory by colposcopy, electrocoagulation is performed. If the abnormality is "suspicious" (i.e., not positively malignant and not positively non-malignant), vaginal, ectocervical and endocervical smears are performed. If the smears are Class III, colposcopical and cytological examinations are repeated every three months. If the smears are Class IV or V a large biopsy is performed under control of the colposcope, and serial sections are done only if necessary.

B. CORNELIS HOPMAN, Miami, Florida, U. S. A.:

To Bajardi: I fully agree with Bajardi that cytology is necessary in every case of a suspicious cervix, before a biopsy is done. The biopsy can easily miss the lesion and this occurs much less easily with a cervical scraping that covers the whole cervix. On the other hand, if there is a suspicious lesion, biopsy should never be omitted, because cytology may give a false negative result. This may occur especially in far-advanced cancer cases with much secretion, infection and bleeding, where fluids may wash the cancer cells away from the cervix or cause so much dilution of the cells on the slides that cancer cells are very scarce or are covered with leukocytes or red blood cells.

To Maria Kawecka and co-workers: Maria Kawecka et al. gives a very good review of cytologic and pathologic results in 20,000 patients with erosion. The cytologic false-negative results of less than 2% are about the same as found in other medical centers, but the overall conformity of cytology and biopsy is better than found in most other laboratories (551 positive first cytology and 519 positive first biopsy in 561 cancer cases). The significance of cytology is further stressed by the fact that in 429 cases no macroscopic evidence of cancer was found, cases which could not have been found by other than cytologic studies. It is possible that some of them could have been found by colposcopy, which is not mentioned by Maria Kawecka et al. Further, the results show again that cytology is more accurate in early cancer and biopsy is more accurate in advanced cancer. In any case this excellent study proves again that prior to biopsy cytology should be performed.

To Kremer: That 37 of 132 cases of cervical cancer were not suspected, as reported by Kremer, is in general accord with our findings. In another series of cases Kremer even found that 51 out of 115 cancer cases were unsuspected. I agree that biopsy could never have been successful in these unsuspected cases, the gynecologist being in the dark as where to do the biopsy. And it is impossible, of course, to do a ring-biopsy or conization on every woman entering the gynecologic clinic. If there is a target lesion, visible tumor or ulceration, I would prefer to do first a cervical scraping for cytology and then a biopsy of the target lesion simultaneously. In advanced cancers, cytology may miss the diagnosis because of bleeding, infection, etc.

To Stoll: I agree with Stoll that cytology and biopsy are necessary for patients with a target lesion. A red spot around the cervix may cover an unsuspected cancer, as Stoll so well described, and should have conization with serial sections, if cytology remains positive. In the line of action for the diagnosis of cervical cancer I would prefer cytology to precede the iodine test, palpation, etc., in order to prevent cells important for the cytologic diagnosis from becoming lost during the examination.

To Song: Song stresses again the importance of cytology in cervical lesions. He advocates the use of scraping smears of the cervix before biopsy procedures are done. I fully agree.

WOLFGANG KORTE, Bonn, Germany:

This question is put precisely. In my opinion it can be answered precisely. If it is possible to carry out both histological and cytological examinations, I shall always perform a biopsy (cervical excision) and cytology.

To Song: Many clinicians are not as afraid as it appears to Song. If one is in the agreeable situation of having been trained for years in pathology, in anatomy and also in gynecology, then one becomes more cautious. After all, the patients do not necessarily live for supplying cell or tissue material for the laboratory, but want to go home calmly and in good health.

We perform the biopsy, which, according to classical principles, should always be taken from as wide a tissue section as possible from the pathologically changed region, as well as from the surrounding healthy tissue. This also has definite practical reasons, since we are then instantly able to perform at the time of biopsy, treatment of the ectocervix by plastic operation or conization. If the material is not suspicious, the patient is set at ease. Moreover, we take the smear on the same day as the excision and have the possibility of cytological controls. If the smear and the biopsy are suspicious or definitely malignant, a more extensive therapy has to be performed.

I am not of the same opinion as Kremer that the biopsy is an insufficient diagnostic provision in the case of a suspicious erosion. As Bajardi remarks, we should, however, not "have to depend upon a small biopsy." These are problems which were acute 50 years ago. In order for the smear to be useful to the cytologist some prerequisites must be fulfilled beforehand, if the results are to be correct. The same is true for the biopsy. The correctly performed biopsy does not belong to the doctor's office. Who would deny the fact that cytology is superior in this respect?

One should, after all, avoid making two valuable examination methods the playthings of competing statistics. It is much more difficult than one might think to make exact comparable extensive examination series.

We always take a biopsy of a clinically manifest ("macroscopically evident") carcinoma of the cervix. But we take this biopsy immediately before the final therapy, i.e., in the same session. The "quick freezing method" according to Schultz-Brauns, gives an exact confirmation of the clinical diagnosis within ten minutes. The function of histology is, in the main, to confirm and not to discover. The danger of transmitting carcinomatous material through lymph vessels opened by biopsy is very small.

The discussion has become somewhat broader than the topic. But I think this is useful. It is my opinion that one can agree with the demands of Stoll. He utilizes all diagnostic methods for the suspicious ectocervix.

Along with the four colleagues from Poland and Bajardi, I think it is excellent that the methods complete or even control each other to a degree.

ERNST-HELMUT KRÜGER, Halle/Saale, Germany:

In general, I agree with the statements of the main speaker. However, I believe that with the appreciation of the diagnostic methods for evaluation of a suspicious cervix, colposcopy has received too little attention. In our opinion the colposcopical picture of the uterine cervix offers to the skilled many hints which allow for the differentiation between a benign and a carcinogenic epithelium. Reviewing our experience we can say that a colposcopically assessed degree of epithelial atypia has almost always been confirmed by histology. In addition a carcinogenic tissue has never been overlooked. The colposcopical suspicion then can always be confirmed by cytology. Very often in such cases of suspicious cervices an additional cytological evaluation remains unnecessary, due to the suspicious colposcopical findings.

WARREN R. LANG, Philadelphia, Pennsylvania, U. S. A.:

If a cervix is "suspicious," it should be biopsied. This is true in spite of any preliminary adjunctive test such as cytology which may have been reported as negative.

Whether or not a cervix is grossly suspicious depends on the examiner, his interest, his past experience and his criteria of abnormality. Evident friability of the portio or the endocervix, ulceration, or proliferation always deserve histologic examination. A so-called erosion is suspected if it is irregular, bleeds easily, occurs away from the os, seems to increase in size, does not heal after cauterization, or occurs in the postmeno-

pausal era when we expect the portio to be covered solely by squamous epithelium. Leukoplakic areas should also be biopsied.

In pregnancy the cervix undergoes proliferation of both squamous and glandular epithelium, especially the latter. This, together with edema, hypertrophy, increased vascularization and even decidual change, often leads the unwary to expect greater histologic abnormality than is actually present. However, here again, biopsy should be performed without hesitation should the observed findings seem unusual or pronounced.

At times the cervix may appear negative and an ancillary examination such as cytology or colposcopy indicate atypism. There is no question then as to what should be done. Maria Kawecka et al. have advised periodic follow-up examinations on all Class II cases. This is certainly desirable, since the borderline between Class II and Class III smears is not always a definite one. The systematic approach of Stoll to the study of the cervix is excellent and represents an ideal plan for discovering early lesions.

J. PAUL PUNDEL, Luxembourg, Luxembourg:

The experience of many gynecologists and pathologists has proved two important facts:

1. An initial punch biopsy taken from a suspicious cervix without control by cytology has frequently given a false negative diagnosis, which in many cases has been accepted as definite by the physician until the cancer arrived at such a degree of evolution that it finally convinced the doctor, but by then it was too late.
2. Early cancers often develop in non-suspicious areas of the cervix or are not seen at the usual gynecological examination.

Therefore, the question asked for this discussion can be answered, a priori, with no.

In a patient showing no visible abnormalities of the cervix, but a positive smear test, biopsy has to be performed, at best by conization of the cervix. If the cervix presents any visible abnormal area, a punch biopsy should be taken at this place. If biopsy and smear are negative, the case can be considered as negative for cancer. If the first punch biopsy is negative, but the smear is or remains positive, conization or even amputation of the cervix should be performed in place of further punch biopsies. If the first punch biopsy shows only a suspicious lesion, or a borderline case or even a carcinoma in situ, I prefer to clear this case immediately by amputation of the cervix in order to obtain complete material for serial histological examination. Personally I do not take a cervical biopsy without a simultaneous cytological smear test, but I would accept one exception as suggested by Kremer, the macroscopically evident carcinoma of the cervix.

## CLOSING REMARKS

HANNES KREMER:

Some of the discussants agree that the value of cytology and of the conization or amputation of the cervix, in cases of positive cytological reports, is beyond all question.

# A COMPARISON OF BLIND ASPIRATION OF THE VAGINAL FLUID AND ASPIRATION UNDER VISUALIZATION

JEAN L. JOHNSON AND TED LACH
Buffalo, New York, U.S.A.

This is a series of smears taken on 22 patients examined in the Out-Patient Clinic at Roswell Park Memorial Institute. Three specimens of cytologic material were obtained from each patient. First, a vaginal smear was taken by blind aspiration of the fluid from the vaginal pool; secondly, a speculum was inserted without lubricant and a second aspiration obtained under visualization; thirdly, a cervical scraping was taken.

All smears were examined for the presence of tumor cells and a comparison of the cell distribution was made by counting 100 consecutive epithelial cells on each slide.

Of the 22 smears obtained by each method, 6 blind vaginals, 6 visualization vaginals, and 5 cervical scrapings were unsatisfactory for counting. In most of these cases, the material on the slide was degenerate and accurate identification of cells impossible.

There were 11 cases in which all 3 smears could be counted. These are shown in Table I where the percentage of superficial, intermediate and basal cells is shown in that order. All of these smears were negative for tumor cells and the distribution of cells showed little variation.

Of the series of 22 cases, only two had positive smears. In one case, many malignant cells were present in all 3 specimens, but all were unsatisfactory for counting. In the second case, only the blind vaginal could be counted, although numerous tumor cells were seen in all 3 specimens.

In conclusion, no significant difference was observed between the smear obtained by blind aspiration and the one taken under visualization. There seems to be little advantage in inserting a speculum to obtain material from the vaginal pool when an equally accurate picture of the cell population may be obtained by blind aspiration. The latter technique may be preferable as it may be preferred by a technician.

Table I

A Comparison of the Superficial, Intermediate and Basal Cells in Blind Aspiration, Aspirations Under Visualization, and Cervicial Scrapings

| Case No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Blind aspiration | 8,89,3 | 1,99,0 | 9,85,6 | 12,81,7 | 7,58,35 | 4,88,8 | 2,36,62 | 1,99,8 | 1,85,14 | 2,98,0 | 4,94,2 |
| Aspiration under visualization | 20,79,1 | 3,96,1 | 5,91,4 | 6,88,6 | 2,48,50 | 6,50,44 | 1,10,89 | 1,68,31 | 1,41,58 | 1,98,1 | 6,92,2 |
| Cervical scraping | 26,70,4 | 4,92,4 | 8,87,5 | 13,74,13 | 10,58,32 | 4,91,5 | 1,11,88 | 2,49,49 | 1,54,45 | 6,94,0 | 4,93,3 |

*Symposium B*

# ENDOCERVICAL ADENOCARCINOMA

## HISTOMORPHOLOGY OF ENDOCERVICAL ADENOCARCINOMA

JEAN A. de BRUX AND
MICHELYNE ANCLA
Paris, France

A survey of the classical works on the subject is striking by the paucity of interest shown by the majority of the authors in the histogenesis of this variety of cancer. The textbooks on pathology devote hardly more than a few lines to this question, and gynecologists like Limburg have simply given a description—very complete, it is true, but strictly morphological. For the past several years, however, the German authors (Gosch, Huber), the Dutch authors (Stoll, Kniekamp, Buttenbert, Döderlein), the American authors (Novak, Hepler, Gusberg), and Swiss authors (Hoffstatter) have directed their efforts toward recognizing, in certain of these adenocarcinomas of the cervix, a mesonephrotic origin. Unfortunately, the authors base their hypotheses only on comparisons with certain renal or ovarian tumors, or, in a few rare cases, on connections between the tumor and the mesonephrotic vestiges or Gartner's duct.

Our experience of adenocarcinomas of the cervix includes 30 cases, observed between 1952 and 1960. Their comparative study, both within this group itself, and between these, and carcinomas from the genital tract in general and the Wilm's tumors, has led us to attempt a classification of the adenocarcinomas of the cervix.

This classification is largely inspired by that which we use for the uterine tumors and that which we are trying to establish for the ovarian tumors, based on their embryogenesis, as have done Cushing and our teacher Percival Bailey for the cerebral tumors. It is not our intention to take up here all the points in detail, as a more extensive work on the subject will appear in another journal. Here we shall simply set forth the different varieties which seem to us to describe particular morphological entities.

Thus, we have divided the adenocarcinomas of the cervix into 3 large classes:

I. Adenocarcinomas arising from adult endocervical structures

Certain carcinomas may reproduce the fringes of the endocervix. Their aspect is papillary, forming macroscopically a bouquet with multiple ramifications. Microscopically, this group shows a frail stroma with a connective axis; sometimes the stroma appears more edematous.

The border epithelium is columnar. The cells have a prominent nucleus, rather voluminous but regular, and a cytoplasm often rather large, and cyanophilic. The Nuclear-Protoplasmic ratio is slightly modified, and anomalies are rare or practically absent. The general aspect is usually that of a hyperplastic endocervical cell, but may sometimes be considerably modified, the cells becoming larger, assuming a puffy, pluristratified aspect. This enlargement involves especially the cytoplasm, which then becomes very eosinophilic, whereas the nucleus remains unchanged and appears less hyperplastic. Hence, the elements shed from such a growth resemble more those exfoliated from an active endocervical hyperplasia than from a carcinoma (Figs. 1 and 2).

Other forms show a marked tendency to reproduce the glands of the endocervix. Sometimes they take the form of lobulated masses crowned by large cells with vacuolized cytoplasm, whose aspect at times assumes a glanduliform aspect and whose nucleus is very voluminous and irregular (Figs. 3 and 4). The secretory character is usually only slightly marked, but there exist forms in which this character dominates the whole histological picture. The pseudo-glandular formations are dilated; the border-cells form papillae projecting into the lumen; they are filled with mucus, and the PAS is strongly positive. At certain points the glandular ordination disappears, and there are found only sheets of mucus-secreting cells. Finally, in certain cases the picture is distinctly that of a colloid mucus cancer, similar to that of the digestive or ovarian cancers (Figs. 5 and 6).

But most often the glandular and papillary aspect of the endocervical cancers are decidedly mixed in with pseudo-glandular forms with apical or basal secretion, occurring in the walls of the papillae as well as in the depth of the conjunctivo-muscular stroma of the cervix. But here again, the cellular anomalies are never very marked (Figs. 7 and 8).

II. Adenocarcinomas arising from the Gartner's duct

On examining serial or semi-serial sections of the cervix, one is struck by the frequency, much greater than as described by the authors, of the canalicular formations—mesonephrotic vestiges more or less dilated, covered by a non-secreting cuboid epithelium with black nuclei in a cyanophilic cytoplasm of little extent. These vestiges are situated in the external half or third of the cervix.

From these forms arise carcinomas of canalicular type, whose elements reproduce the morphological characters of the Gartner's duct, without ever assuming the secretory and hence clearly distinguishable charac-

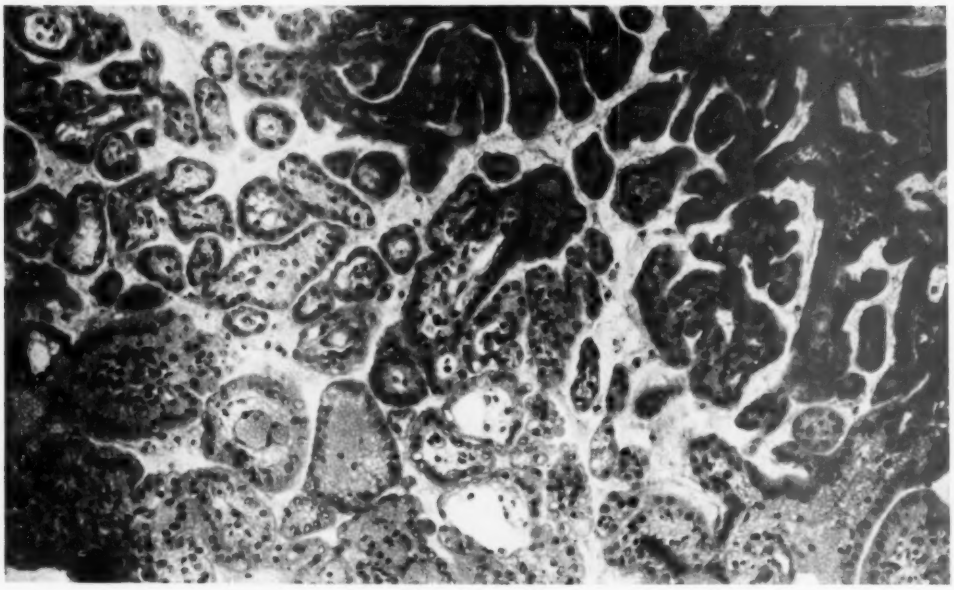


Fig. 1. Endocervical adenocarcinoma, pseudo-polyp type.

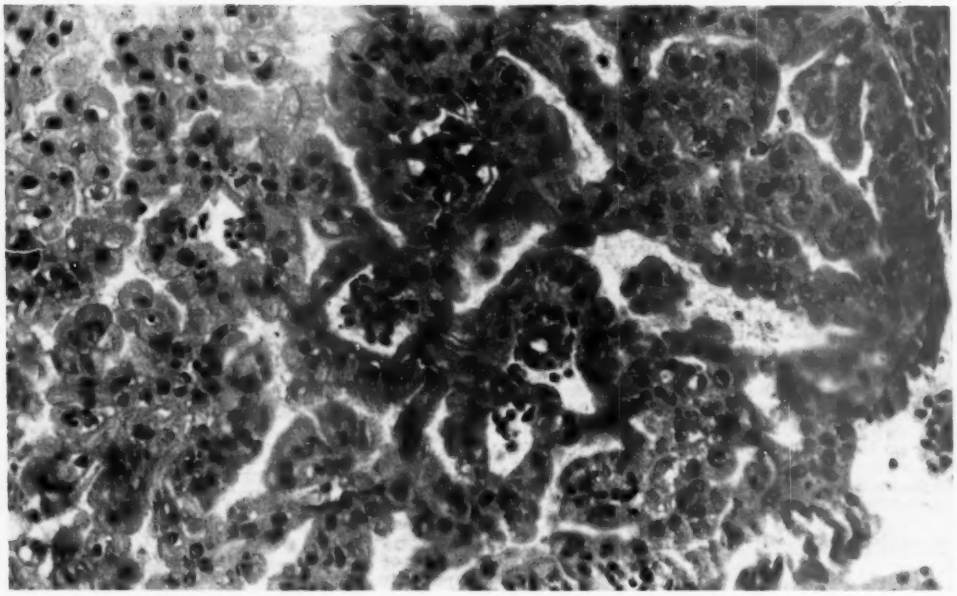


Fig. 2. Adenocarcinoma of the cervix of pseudo-polyp type area with eosinophilic metamorphosed cells.

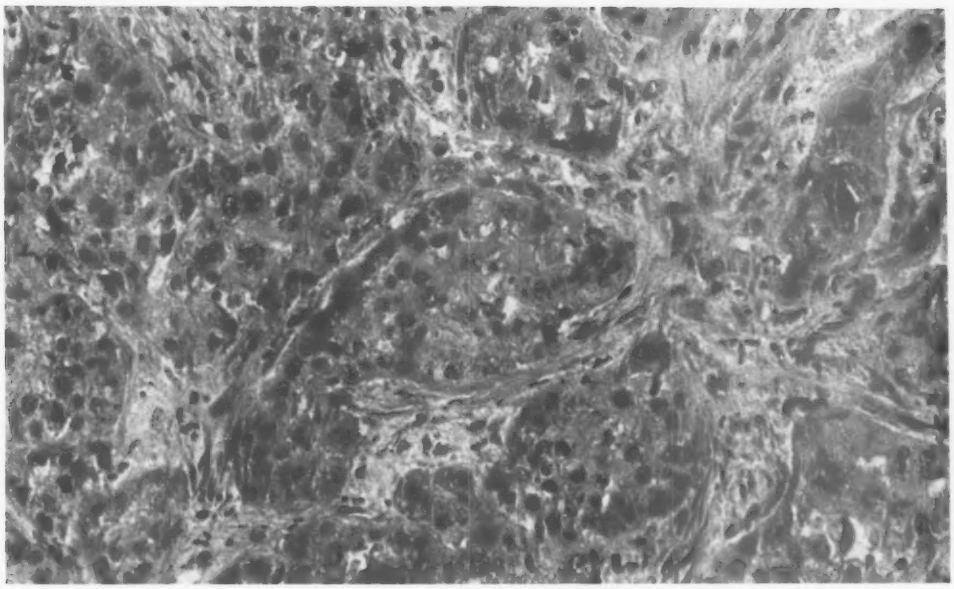


Fig. 3. Typical adenocarcinoma of glandular type.

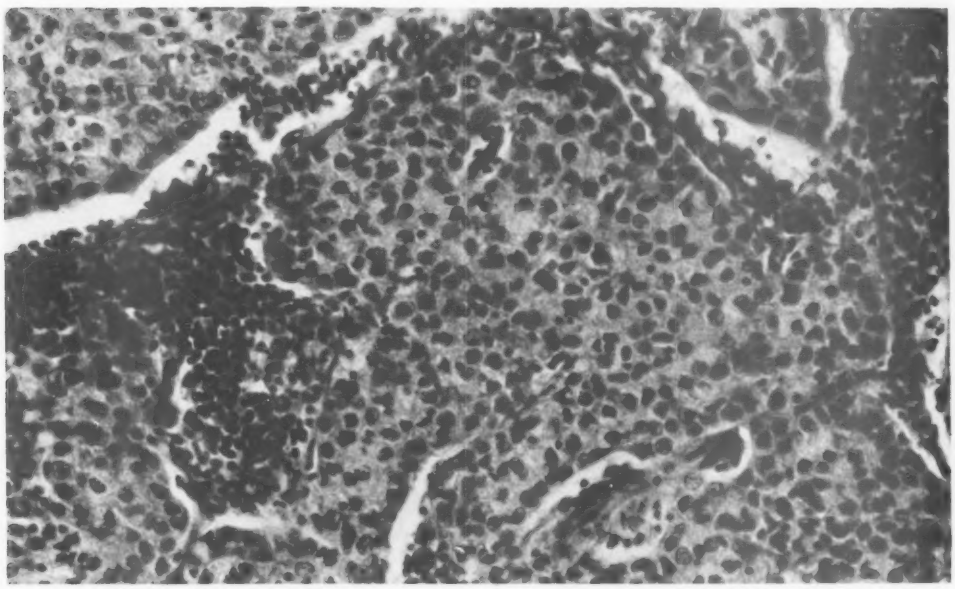


Fig. 4. Adenocarcinoma of glandular type with monomorphous wide invasive area.

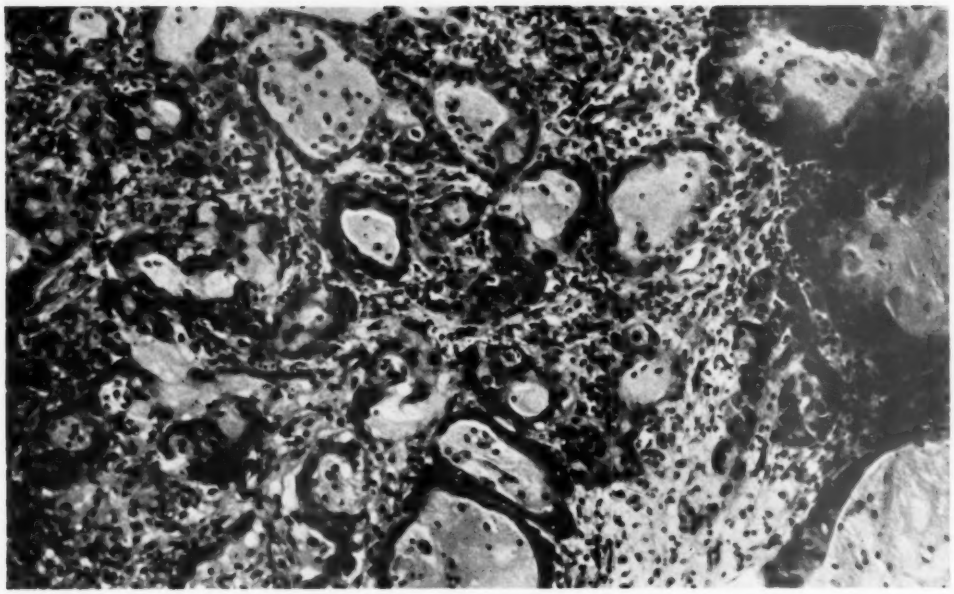


Fig. 5. Endocervical adenocarcinoma of pseudo-glandular type with mucous-secreting cells.

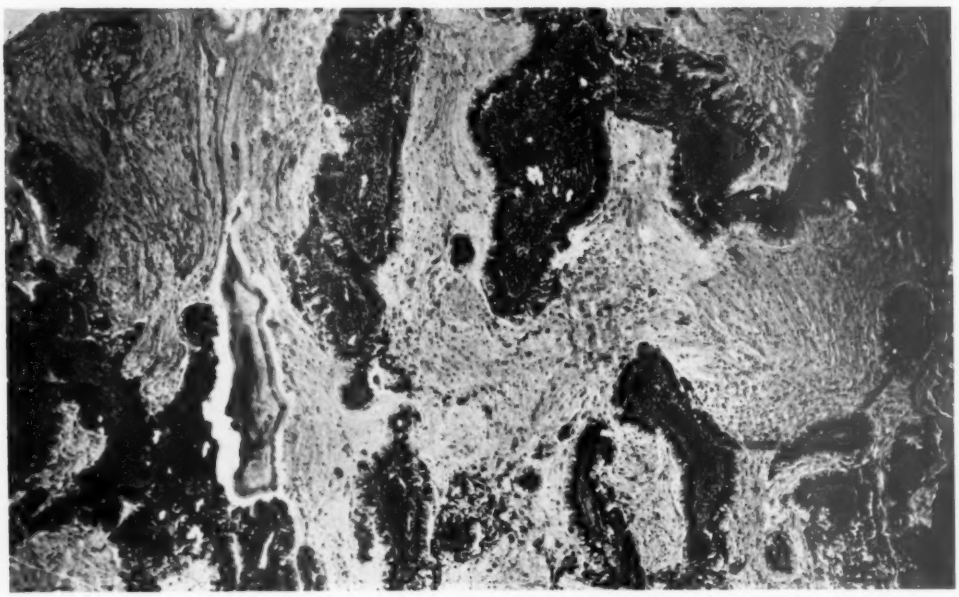


Fig. 6. Adenocarcinoma of mucous-colloid type.

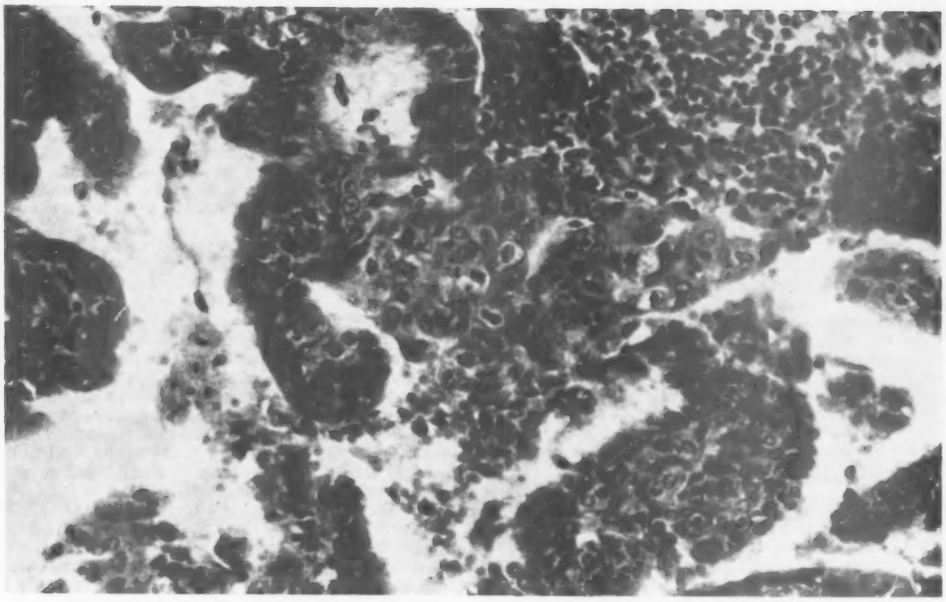


Fig. 7. Endocervical adenocarcinoma of pseudo-polyp and pseudo-glandular type.

Fig. 8. Adenocarcinoma of endocervix of pseudo-glandular and pseudo-polyp type.

ter of the endocervical mucous glands. These newly-formed canalicules, usually all of identical size, are ranged side by side, pushing toward the lower part of the cervix without ever passing beyond the isthmic region, and toward the endocervical canal, crowding back the columnar epithelium, which renders these forms "silent" as long as they have not ruptured the mucosa (Fig. 9).

In addition to this form, we have had the opportunity of observing a particularly rare form, of which Limburg in his book reports only 2 cases—the double carcinoma. This consists of:

A. a canalicular carcinoma of Gartnerian origin;
B. an epidermoid carcinoma replacing the surface epithelium and penetrating into the mucous glands.

These two carcinomas are clearly and visibly distinct, and not an epidermoid metaplasia in an adenocarcinoma of the cervix, as proved by the biopsy and the smears. This double cancerization is as yet very difficult to detect, a single cancerogenic factor having probably induced the double lesion (Fig. 10).

III. Adenocarcinomas arising from embryonic vestiges having multiple inductive potentialities

The extraordinary morphological diversity of the majority of the cervical adenocarcinomas which we have examined has led us to abandon the currently-used classification based on a static morphology without issue, in favor of the pathogenic conception of Robert Meyer, unjustly forgotten.

According to Meyer, the Wolffian canal is always found up until the second or third month of fetal life. From the fourth month on, it gradually disappears. Nevertheless, he found it persisting in 22% of cases in a series of 44 adult uteri. Huffman (1948), in a study of 1,192 cervices, found a much smaller percentage: only five cervices contained mesonephrotic vestiges, and five presented neoformations.

The mesonephrotic duct enters the uterus at the level of the internal orifice, traverses the cervix and penetrates into the vaginal wall. The persistence of the mesonephrotic duct is a proof of the vitality of this epithelium, resisting the physiological process of degeneration. However, some very interesting works on the subject attribute to the Wolffian duct a role of prime importance in the constitution of the female genital organs. Gruenwald, from 1944 to 1952, studied at great length the inductive role of the Wolffian duct in the formation of the Müller's duct. Such authors as Cambar (1948), Nieuwkoop (1948), Fraser (1950), Burns (1955) and Grobstein (1955) have confirmed and defined the role of the Wolffian duct, even that of only a few cells, as being capable of inducing the differentiation of the mesoderm into the mesonephros. Nieuwkoop considers that a very feeble stimulus is sufficient, the threshold of inducting being very low.

These ideas are a necessary basis for an understanding of our classification of certain cervical adenocarcinomas whose characters are far removed from those of the tumors above described, and also for an understanding of the complexity of certain of them, for which a single description is impossible.

The hypothesis on which we build our classification may be resumed as follows: the Wolffian cells, at a particular moment and under the influence of some stimulus, act on the cervical connective tissue, which has conserved a special susceptibility. We may then observe the occurrence of proliferations which will be either:
A. regressive tumors, resembling the mesothelioma;
B. clearly differentiated toward a mesonephrotic type;
C. differentiated from a Müllerian type; or D. extremely complex, consisting of cells grouped in an architecture recalling several of these embryonic tissues.

A. Regressive tumors of mesothelial type. Certain tumors strikingly resemble the malignant mesotheliomas. They are composed of very inter-crossed and ramified fringes bordered by large cells with cyanophilic cytoplasm and bulky nuclei. At certain points these cells are in formations of puffy appearance, at other points they become eosinophilic (Fig. 11).

Sometimes (Fig. 12) these tumors penetrate into the stroma of the cervix, forming cleavages of varying width bordered by extremely voluminous, cyanophilic cells. It is this form which is sometimes found in certain carcinomas of the corpus uteri, and whose prognosis is grave. We also find the same pictures in the complex carcinomas.

We must therefore draw a comparison of this variety of tumor with another closely-related tumoral variety, namely, the mesothelioma of the uterine cervix.

B. Tumors differentiated toward a mesonephrotic type. These tumors are theoretically quite numerous because whenever pathologists meet with a tumor of abnormal morphology they tend to classify it under this term. In reality, no conclusive argument is brought to support this conception, except the comparisons made with certain renal tumors or with certain tumors of the genital zone, in particular, the clear-cell tumors in the ovary and the uterus (Saphir et al.), sometimes brought into evidence (Figs. 13 and 14).

Those which we have been able to study show clear cells with a large, dense and chromatic nucleus pushed against the cytoplasmic membrane. This clear aspect does not indicate a secretory state, but is a sign of cellular suffering. As a matter of fact, we have been able to find all the transitional aspects between cyanophilic and eosinophilic elements disposed on papillary axes or on pseudo-canalicules. The architecture is that of the renal clear-cell tumors. One notes, in fact, frail reticular axes forming the stroma and on which the cells are inserted. The lines are either inter-crossed or parallel, giving an impression of large cellular sheets. Sometimes the cellular degeneration is so extensive that the cells appear dispersed without any ordination. Their nuclei become enormous and chromatic, and in one case the aspect of the cells were so anaplastic that the diagnosis of botryoid sarcoma had been given. The surgical specimen upheld the diagnosis: more deeply-lying, there were found a lobular architecture and an aspect of clear-cell carcinoma (Fig. 15).

However, the carcinomas of nephrotic appearance are not necessarily "clear-cell" tumors. We believe that certain papillary images may have an identical origin. There may be noted, in fact, frail, ramified axes bordered by several superimposed layers of large cells, sometimes eosinophilic but more often cyanophilic. These have a bulky, very irregular nucleus, and their cytoplasm tends to become vacuolated. Sometimes they become organized into pseudo-glands, or disappear by vacuolization of the cytoplasm and progressive lysis of the nucleus, following a process well known in regressive phenomena in embryology (Fig. 16).

Other modifications recalling embryological phenomena are encountered in these tumors. Certain cells show an extremely bloated and very regular eosinophilic cytoplasm, assuming an almost hyalin character. This aspect is very singular and at first may be taken for an epidermoid metaplasia (Figs. 17 and 18). Another modification, no less singular, was encountered in one of our cases, which was the xanthomatous transformation of the tumoral stroma cells. This modification has been pointed out in certain nephroblastomas. In most of these tumors, there should also be noted the "cannibalism" of the tumor cells, which absorb very numerous polynuclear cells. This is a rather characteristic sign of the endocervical carcinomatous cells.

C. Tumors differentiated toward a Müllerian

Fig. 9. Adenocarcinoma of endocervix, canalicular type of Gartnerian origin.

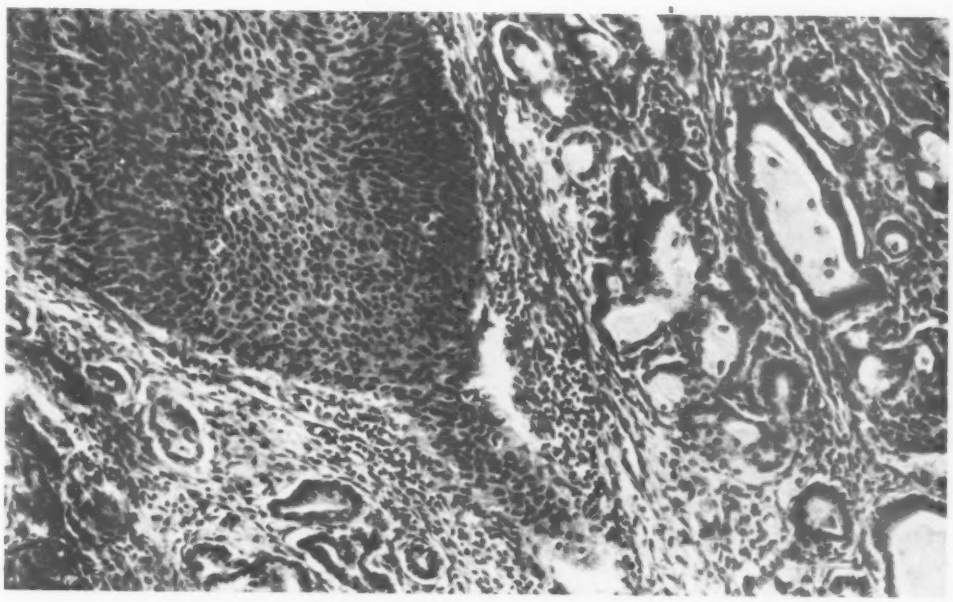


Fig. 10. Double carcinoma of the endocervix, epidermoid type and canalicular Gartnerian type.

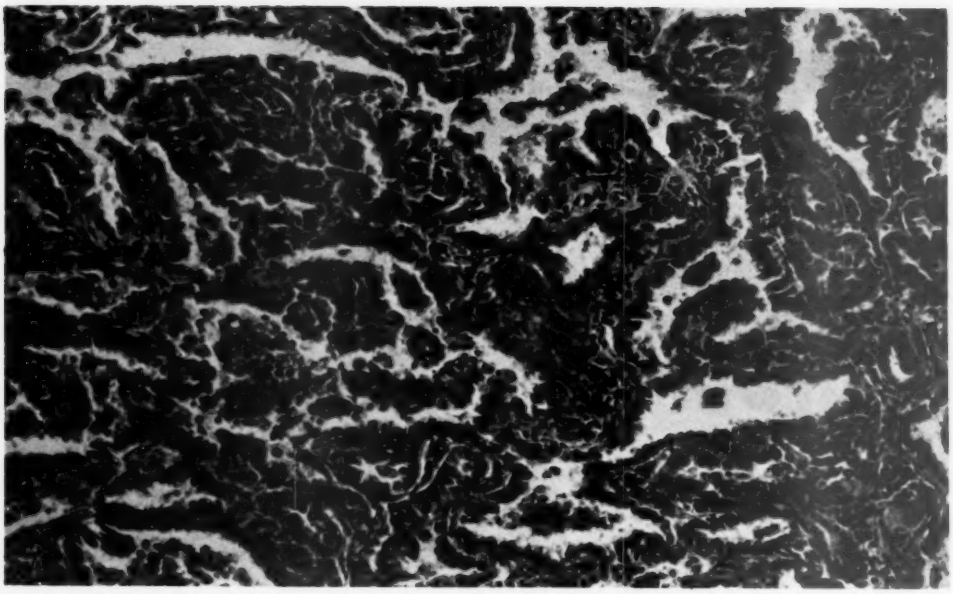


Fig. 11. Endocervical carcinoma of "regressive mesothelial" type.

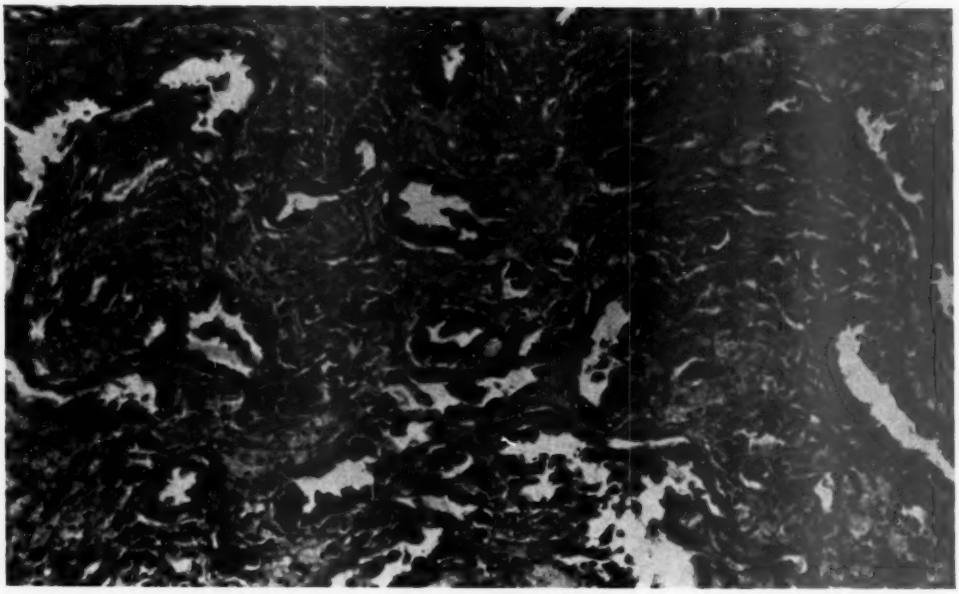


Fig. 12. Endocervical carcinoma of "regressive mesothelial" type invading the endocervical stroma.

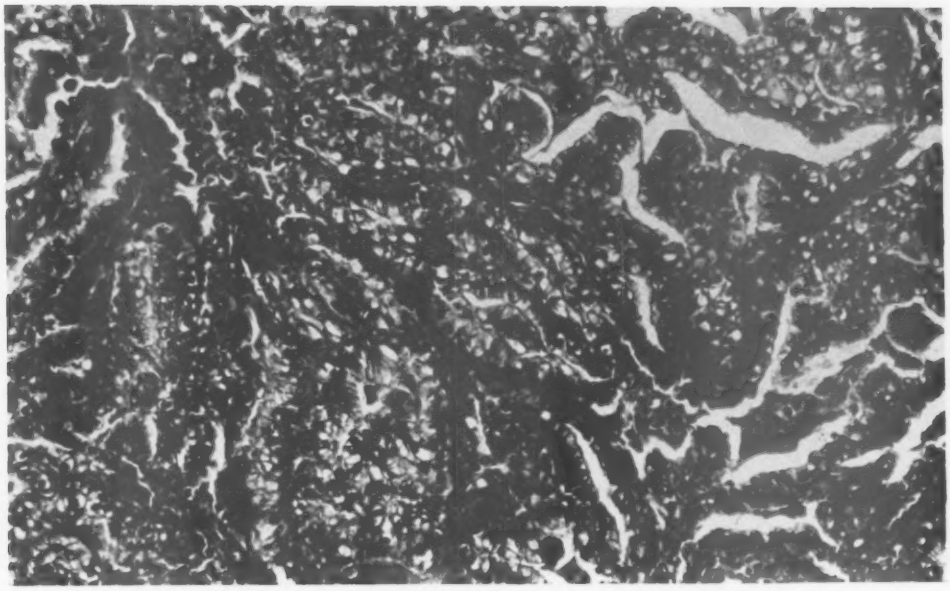


Fig. 13. Endocervical carcinoma of mesonephrotic type (clear cell carcinoma).

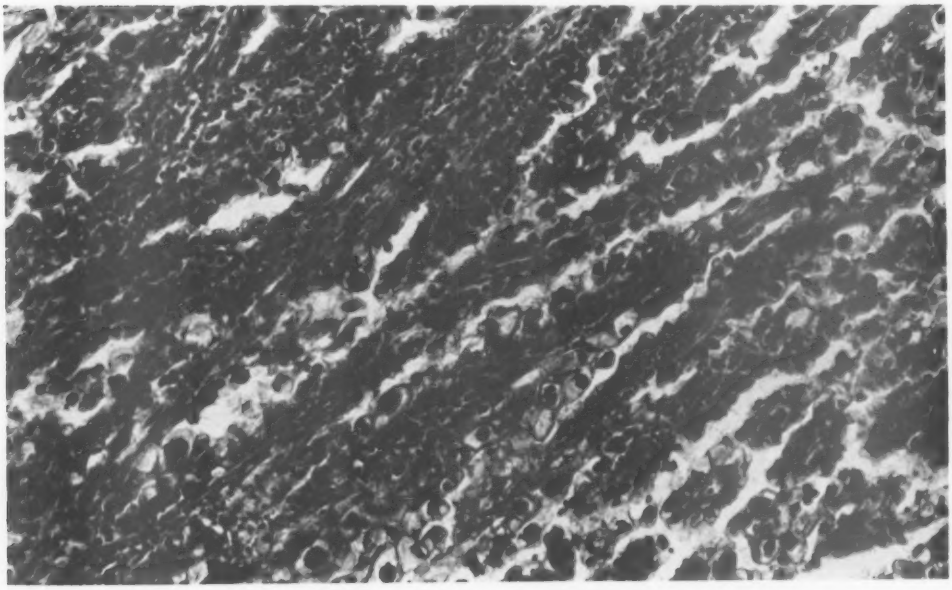


Fig. 14. Endocervical carcinoma of mesonephrotic type (clear cell carcinoma).

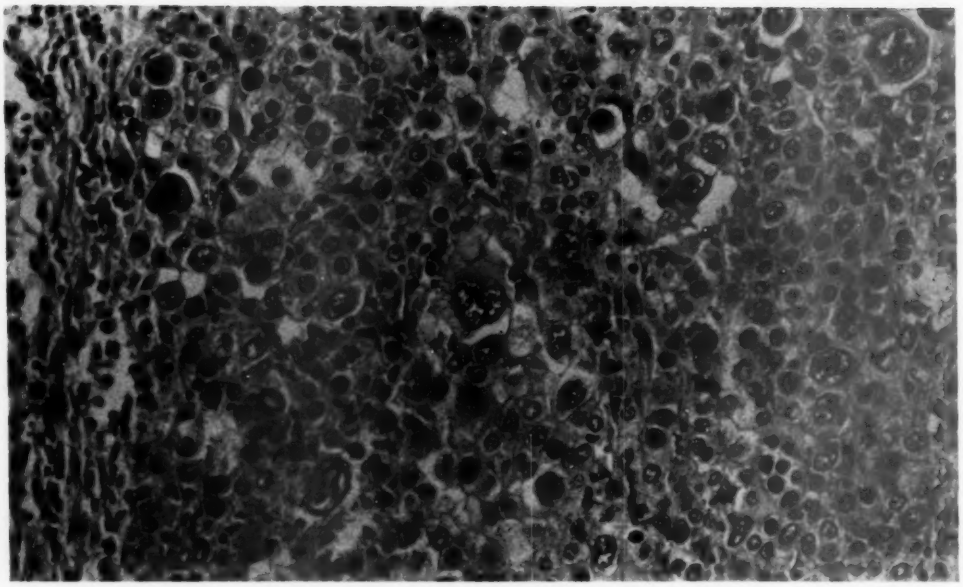


Fig. 15. Anaplastic carcinoma with enormous cells.

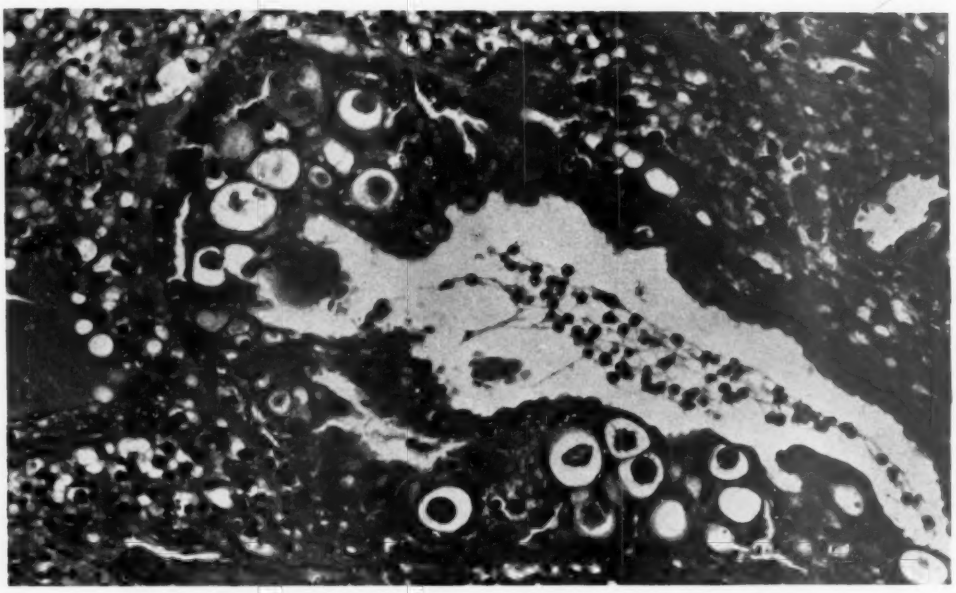


Fig. 16. Endocervical carcinoma of mesonephrotic type (clear cell carcinoma).

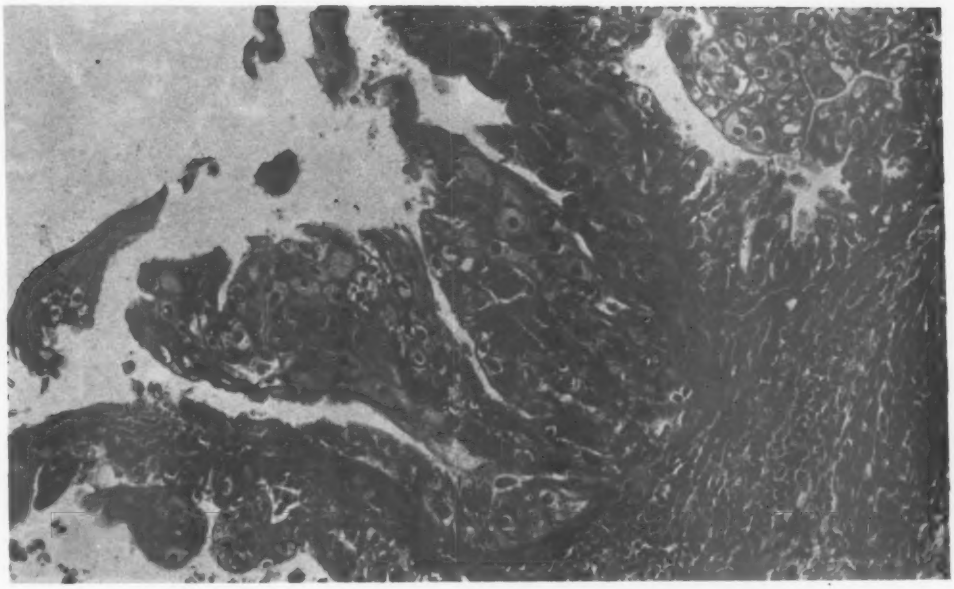


Fig. 17. Endocervical carcinoma with papillary axes and large cellular sheets.

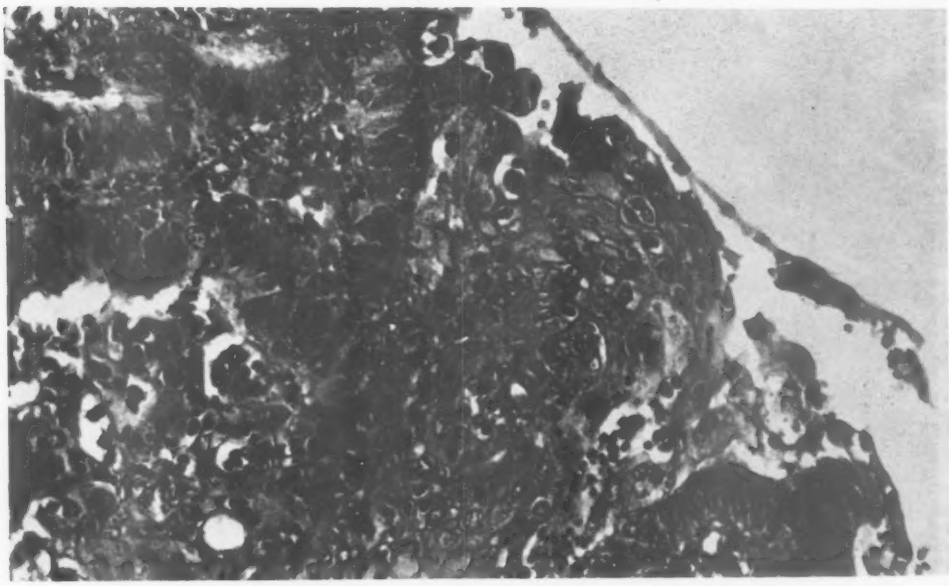


Fig. 18. Pseudo-glandular organization of the adenocarcinoma with progressive lysis of the cells.

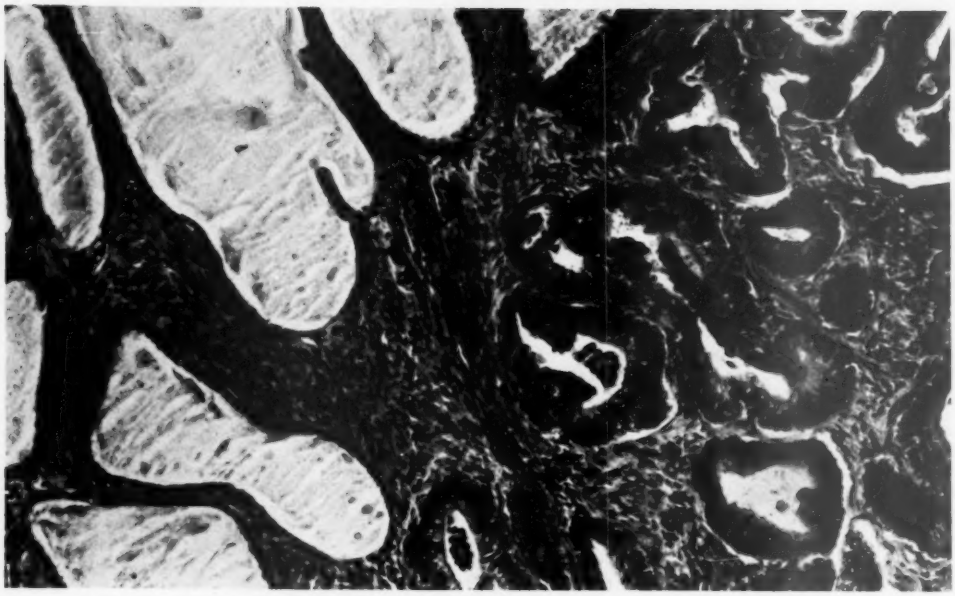


Fig. 19. Endocervical adenocarcinoma of Müllerian type (look at the difference between blown endocervical mucous glands and carcinomatous pseudo-glands).

Fig. 20. Endocervical carcinoma of Müllerian type (look at the peritubular stroma looking like endometrial stroma).

type. The return to embryonic activity of the Wolffian duct may be exteriorized by a neo-induction of the Müller's duct. We were able to study two cases in which the carcinomatous formations had the aspect of an endometrial cancer, to such an extent that this diagnosis had been made on the biopsy. But the surgical specimen showed the major localization at the level of the endocervical wall, with rupture of the endocervical mucosa and infiltration toward the isthmus in the very heart of the connective stroma of the cervix. A noteworthy point was the total integrity of the super-isthmic endometrium (Figs. 19 and 20).

The architecture and the cellular morphology resembling to a deceptive degree those of an endometrial adenocarcinoma. In fact, there were noted glandular tubes packed together, sometimes separated by a typical endometrial stroma. Certain glands took an eosinophilic stain. The glands and the mucosa of the endocervix, pushed back by the tumoral process, showed cellular modifications recalling those encountered in the endometrial zones not yet involved by the neighboring adenocarcinoma.

D. The complex tumors. One form seemed to us to merit being isolated. In this it was possible to recognize several varieties of tumoral pictures of embryonic type: an aspect resembling the mesothelioma, the clear-cell cancer, the mesonephrotic cell with large papillae, and finally a distinctly Müllerian zone.

This tumoral complexity finds an explanation in the hypothesis of the work that has guided our classification and which takes its support from the works of the embryologists and from morphological identities.

The Wolffian vestiges may themselves multiply, but may also, by phenomena of induction, "ignite" a tumoral activity on the connective tissue, which reacts as previously did the embryonic mesoderm, giving tumors of a regressive type. More often, however, the Wolffian vestiges grow to a differentiated type of tumor, either mesonephrotic or Müllerian. But in some cases all the types of cancer are represented in a single tumor.

We are well aware that the last part of our classification is built on a working hypothesis. However, we are on the way to bringing other major proofs in support of this assumption.

## Bibliography


Bailey, P. and Cushing, H.: A classification of the tumors of the glioma group on a histogenetic basis with a correlated study of prognosis, 1926, J. B. Lippincott Company ed.

Burns, R. K.: Analysis of Development, p. 462, 1955.

Buttenbert, D.: Strahlentherapie 112:47-53, 1960.

Cambar, R.: Bull. biolog. France et Belgique 8:214-285, 1948.

Doderlein: Der Zentralb. für Gynäk. 184:39, 1953.

Fraser, E. X.: Biol. Rev. 25:159, 1950.

Gosch: Z. f. GEB 129:103, 1948.

Grobstein, C.: Advances in cancer research, New York, 1956, Ac. Press, 187-233.

Gruenwald, P.: Anatomical Record 81:1-19, 1941.

Gruenwald, P.: Anatomical Record 86:321, 1943.

Gruenwald, P.: Ann. N. Y. Acad. of Sciences 55:546, 1952.

Gruenwald, P.: Ann. N. Y. Acad. of Sciences 82:12-18, 1958.

Gusberg, S. and Corscaden, J. A.: Cancer 4:1066, 1951.

Hepler, Th. K. et al.: Am. J. of Obst. and Gyn. 63:800, 1952.

Hofstatter, R.: Gynaecologia 146:256, 1958.

Huber: Strahlenth. 37:334, 1930.

Kniekamp: Zbl. Gyn. 80:575-580, 1958.

Limburg, H. and Thomsen, K.: Das Adenokarzinom des Collum Uteri, Thieme-Stuttgart, 1949.

Meyer, R.: Z. f. Geb. 58:528, 1906.

Meyer, R.: Z. f. Gynäk. 61:2846, 1937.

Nieuwkoop, P. D.: Experientia 4:391, 1948.

Nieuwkoop, P. D.: J. exp. Zool. 120:1-108, 1952.

Nieuwkoop, P. D.: Exp. cell research supp. 3:262, 1955.

Novak, E., Woodruff, J. D. and Novak, E. R.: Am. J. of Obst. and Gyn. 68:1222, 1954.

Saphir, O.: Obst. Gyn. 9:368, 1957.

Stoll, P. and Buttenbert, D.: Geburt. Frauenheilk. 15: 698-711, 1955.



EMMERICH von HAAM

Columbus, Ohio, U.S.A.


The histological picture of adenocarcinoma of the cervix varies greatly. Not only do we find more differentiated and less differentiated types, but among the more differentiated types various subvarieties can be recognized. Figure 1 represents the histological picture of a carcinoma of the endocervix to which the name "papillary adenocarcinoma" could rightly be attached. Tall columnar cells form epithelial linings around stromal papillae and little evidence of mucus secretion can be noted. This is in quite marked contrast to Fig. 2, representing a section of a tumor known in the literature as adenoma malignum. Here well-formed glands are dispersed throughout the stroma which are secreting copious amounts of mucus. Two other peculiar forms of well-differentiated adenocarcinoma of the cervix are illustrated in Figs. 3 and 4. In Fig. 3 we are dealing with a mucus-secreting carcinoma where small malignant cells form large cystic spaces filled with vast amounts of mucus, whereas in Fig. 4 the mucus remains within the malignant cells, thus producing signet-ring cells not unlike those observed in Krukenberg tumors. As we approach the less differentiated types of tumors the differentiation between the papillary and the glandular forms of adenocarcinoma becomes less distinct. The Grade IV carcinomas are extremely anaplastic and often cannot be distinguished from the Grade IV squamous cell carcinomas.

We have observed in the past four years 19 cases of adenocarcinoma of the cervix which affected women from 32 to 66 years of age. Six of the tumors belonged to the well-differentiated type, 2 were papillary carcinomas, 4 well-differentiated adenocarcinomas. Four were classified as Grade II, all presenting more or less the glandular type of tumor. Four were classified as Grade III, while 3 belonged to the highly undifferentiated group of tumors. In 2 patients adenocarcinoma as well as squamous cell carcinoma was present. One of these cases showed extensive squamous cell carcinoma in situ involving the entire external surface of the cervix with few areas of microinvasion, while in the other case squamous cell carcinoma of Grade III variety mixed freely with adenocarcinoma, Grade II. Two similar cases have been described by Fricke and co-workers (1).

The different pictures obtained in the well-differentiated types of adenocarcinomas of the endocervix would suggest different histological origins of these tumors. No uniform opinion exists as yet concerning the types of cells found in the endocervix. While Faulkner (2) definitely states that all endocervical cells are mucus-secreting, other authors (3,4) suggest the existence of non-mucus-secreting ciliated cells. According to Frost (5), a third type of endocervical cell which is neither ciliated nor mucus-secreting can be found. This could explain the great histological variety found in the more differentiated types of these tumors.

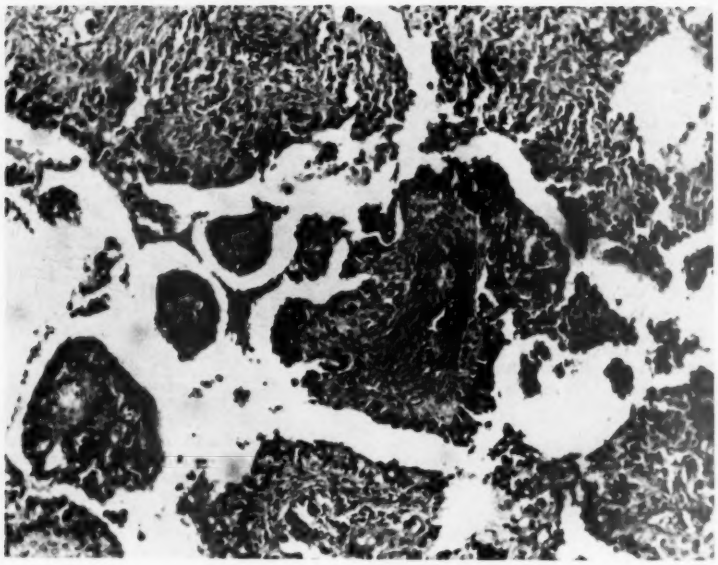


Fig. 1. Papillary adenocarcinoma of the endocervix. Hematoxylin-eosin, X120.

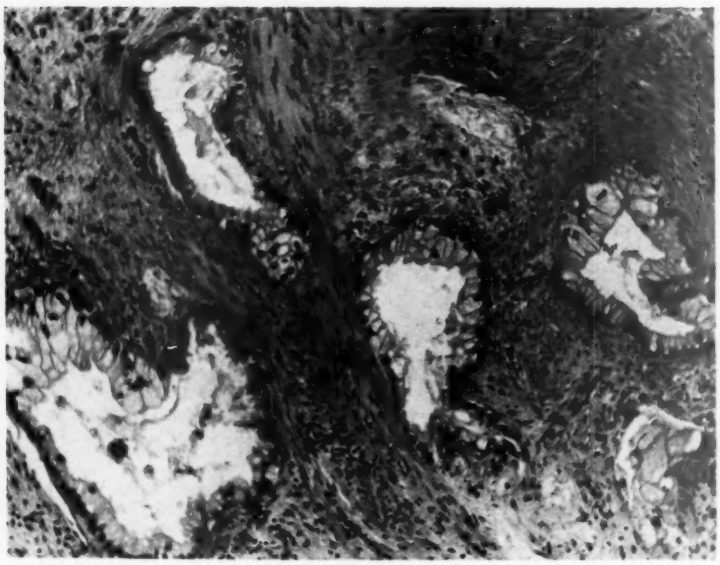


Fig. 2. Well-differentiated adenocarcinoma of the endocervix (adenoma malignum). Hematoxylin-eosin, X120.

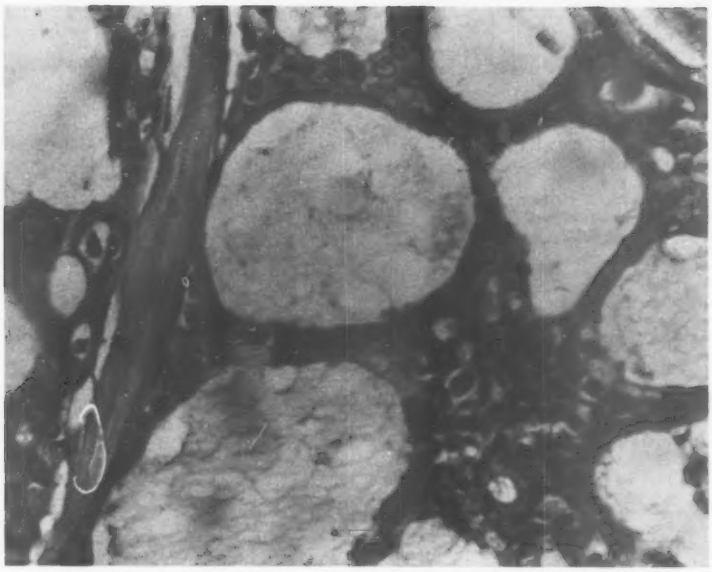


Fig. 3. Mucoid adenocarcinoma of the endocervix. Hematoxylin-eoxin, X480.

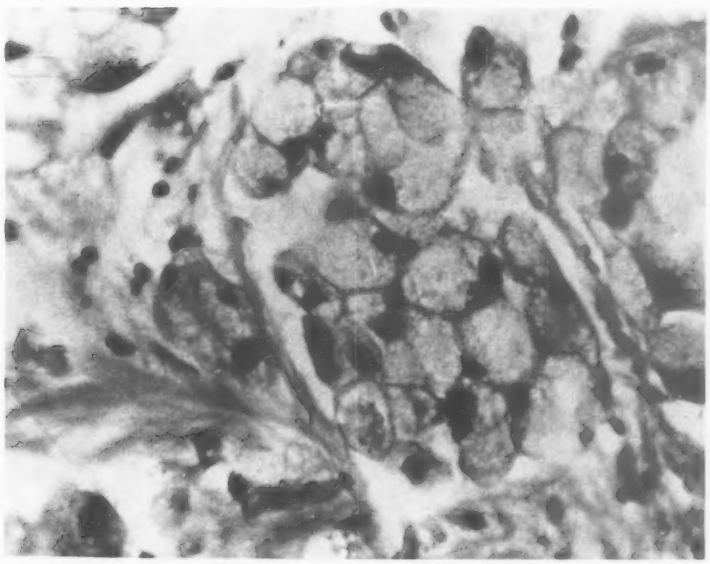


Fig. 4. Mucoid adenocarcinoma of the endocervix with signet-ring cells. Hematoxylin-eosin, X480.

Bibliography

1. Fricke, R. E., Soule, E. H. and Craig, R. M.: J. Am. Ger. Soc. 4:113, 1956.
2. Faulkner, R. L. and Douglass, M.: Essentials of Obstetrical and Gynecological Pathology, ed. 2, St. Louis, 1949, C. V. Mosby Co.
3. Borst, M.: Pathologische Histologie, Leipzig, 1926, F. C. W. Vogel.
4. Smolka, H. and Soost, H. J.: Grundriss und Atlas der Gynäkologischen Cytodiagnostik, Stuttgart, 1956, Georg Thieme.
5. Frost, J. K., in Novak, E. and Novak, E. R.: Gynecologic and Obstetric Pathology, Philadelphia, 1958, W. B. Saunders Co.

RAIMUND KRIMMENAU

Dresden, Germany

The primary adenocarcinoma of the endo- and ectocervix is far rarer than the squamous carcinoma. Novak indicates that this ranges from 2.7 to 13.5% of all cervical carcinomas (5). With a progressive tumor formation at the ectocervix and in its vicinity, the proof of a primary cervical carcinoma cannot be furnished with certainty since there exists the possibility of a spread downwards from the corpus or a spread inwards from the ovaries or even metastases to Douglas pouch of extragenital tumors. Such stages are outside of the study of cervical adenocarcinoma.

The question arises of the genetics of undifferentiated cells of the cervical area of the glands and, rarely, mesonephrogenic remnants (Gartner's duct, which according to Robert Meyer persists in 20% of the cases). We found such remnants also in serial sections of conizations.

From 1956 to the present the material of certain cervical adenocarcinomas is at our disposal in the form of conizations or surgical specimens. During this time we found 3.7% (14 cases).

According to the predominant outward appearance they can be distinguished as tubular, alveolar, papillary, or gelatinous (Fig. 1). With the so-called highly differentiated forms glandula mimetic growths are found, which still closely resemble the normal cervical glands (adenoma malignum). The epithelium is hardly ever single layered. Mostly there is found a many layered epithelium with large nuclei rich in chromatin, with polymorphism and mitoses, also atypical mitoses. The epithelial proliferation leading to the formation of solid positions of the tumor is generally considered the immature form. The capability to produce mucus changes in its extent (mucicarmine and PAS-positive, sometimes also Best-carmine-positive, contrary to the cervical mucous glands showing only in rare cases such reactions) (Fig. 2). Expansion of the lumen with penetration of mucus to the connective tissue may occur and can give the neoplasm its characteristic appearance (carcinoma gelatinosum). Recently we saw an ectocervical tumor composed of complexes of large and clear cells with an only partly existing narrow lumen, so that a hypernephroid picture arose. Mucus-staining procedures were positive (Fig. 3). It should be realized that formation of mucus is no proof of cervical origin (Fig. 4). Not so rare is the finding of a more or less pronounced mucus formation in corporal adenocarcinomas. Squamous epithelial

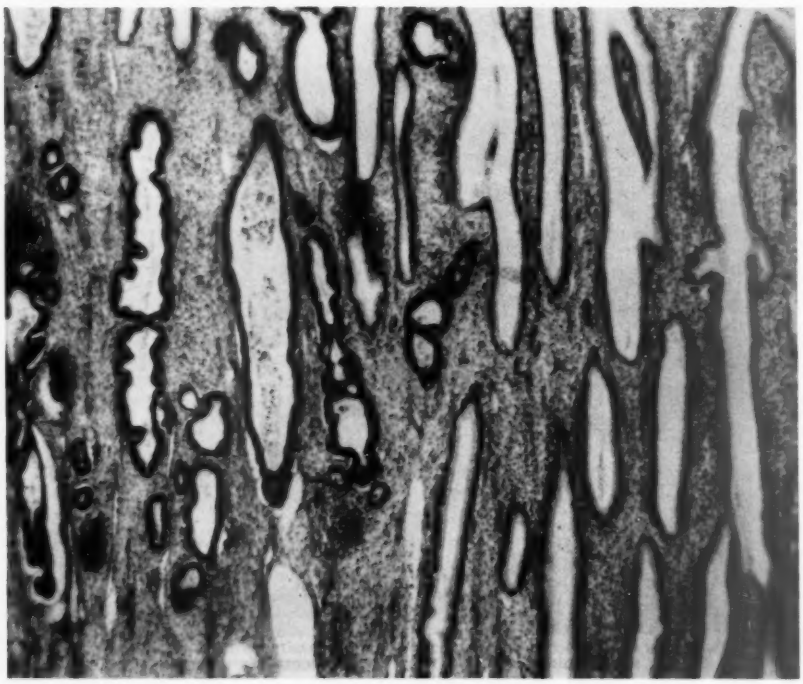


Fig. 1. Fully developed tubular adenocarcinoma with partly single-row, dark glandular epithelium (left). On the right, normal cervical glands.

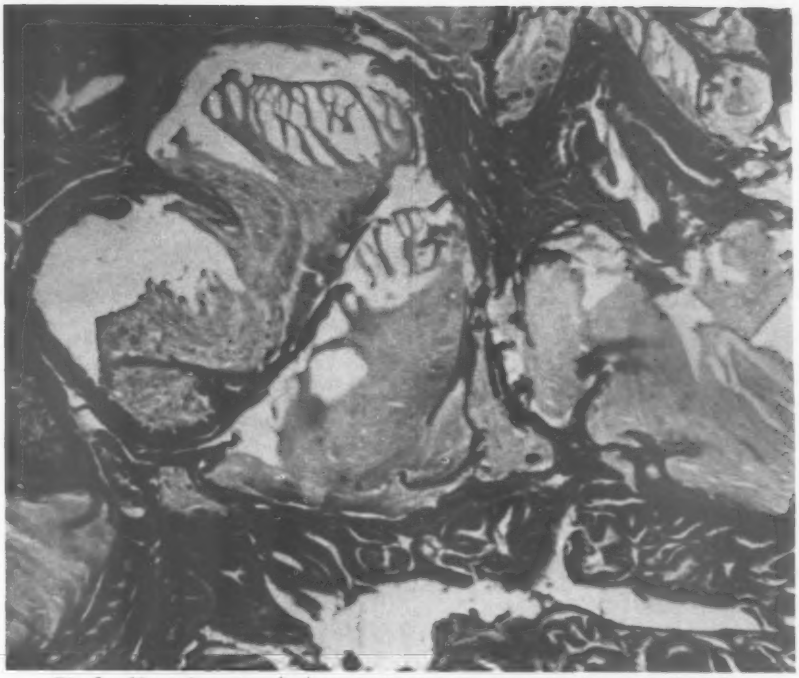


Fig. 2. Mucus formation (top) penetrating into the connective tissue. On the left one still recognizes in the mucus a seam of the epithelium, otherwise only fibrous septa. Below, carcinomatous glandular complex.

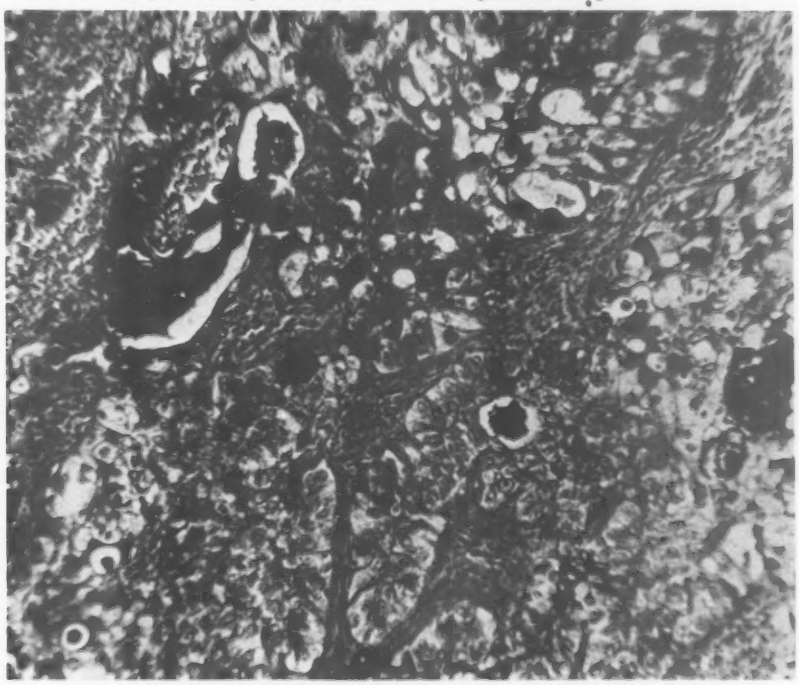


Fig. 3. Large-cell, solid adenomatous mucus-carcinoma. PAS-staining. Mucus appears black in the picture.

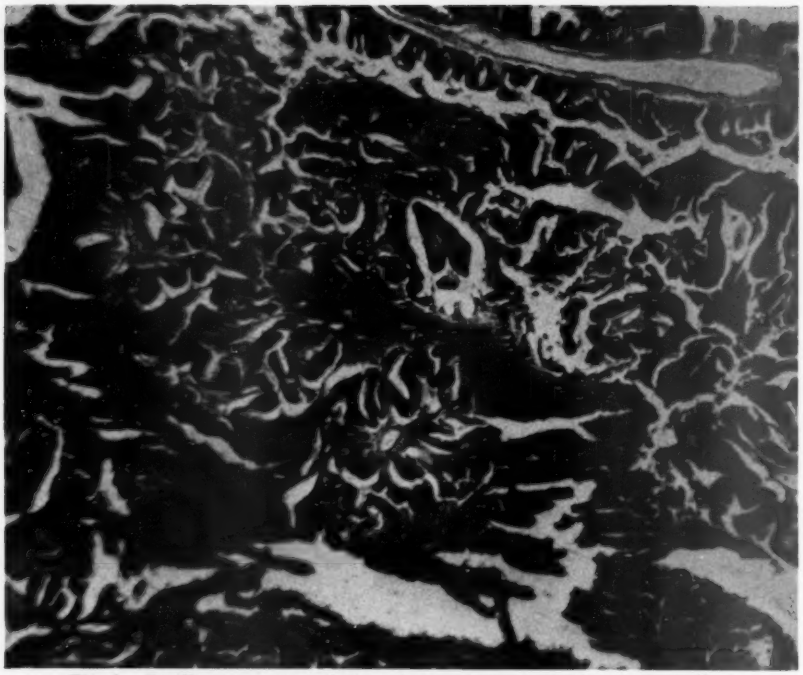


Fig. 4. Papillary, mucus-producing adenocarcinoma as it also occurs in the corpus. Mucus between the tumor parenchyma and in the epithelium. Here appearing black, PAS-staining.

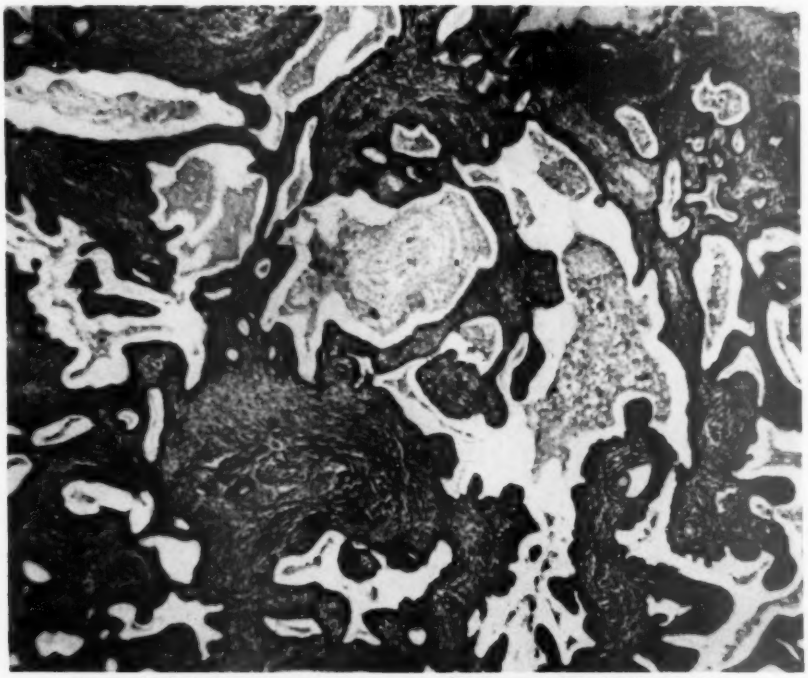


Fig. 5. Endothelioma-type adenocarcinoma (the tumor infiltrated the total anterior wall of the cervix and was solidly diffused other places. An excision three months ago was diagnosed elsewhere as a "polyp").

nodules as with glandular hyperplasia and corporal carcinoma were also in this case called an adenocancroid (adenoacanthoma).

If a solid or diffuse growth predominates differentiation may become difficult, since a formation of lumina also occur in squamous cell carcinomas. Wide, irregular formations of lumina lined by a low, flat epithelium formerly were described as "endothelioma" (Fig. 5). For tumors of Gartner's duct a coherence with remnants of it is demanded by Robert Mayer. However, as publications show, this frequently cannot be produced. Limburg, Thomsen, Schmitt and Stoll (3,7,8) emphasize the abnormal structure of the cervix: small-tubular overgrowth of the glands with uniformly low epithelium, partly solid proliferative partions and possibly positive glycogen reaction. In the differential diagnosis one may consider in this case inflammatory cervical gland proliferations (Limburg, Thomsen (3), R. Meyer (4)). The importance of these tumors is considered differently (8) (Fig. 6). Norris described tumors with scanty parenchyma and highly developed stroma (6). Adenocarcinomas of the cervix are found in the cervical canal and on the ectocervix and apparently are now detected earlier by means of colposcopical and cytological examinations. Tumors attributed to mesonephric origin are located, in general, in the lateral portions of the cervix.

Bibliography


1. Dubrauszky, V.: Grundriss der path. Anat. u. Hist. d. weibl. Geschlechtsorgane. München, 1954, I. A. Barth.
2. Huffmann, J. W.: Am. J. Obst. & Gyn. 56:23, 1948.
3. Limburg, Thomsen: Das Adenocarcinom des Collum uteri. Stuttgart, 1949, G. Thieme.
4. Meyer, R.: Handb. d. spez. path. Anat. u. Hist. von Henke–Lubarsch, Band VIII/1. Berlin, 1930, Springer.
5. Novak, E.: Gyn. and Obst. Path. Philadelphia, 1956, Saunders.
6. Norris, C. C.: Am. J. Cancer 27:653, 1936.
7. Schmitt, A.: Gebh. u. Frahde 11:505, 1951.
8. Stoll, P.: Gebh. u. Frahkde 10:219, 1950.


## DISCUSSION

CONSTANTIN HEROVICI, Villejuif, Seine, France:

The three main papers on the morphology of adenocarcinoma of the endocervix by Drs. de Brux, von Haam and Krimmenau are very interesting and show us the complexities of the tumors with their histo-embryologic connections. The three speakers classify these tumors using embryological classifications. In this regard, the paper of de Brux is particularly interesting. One asks oneself whether or not one will be better able to characterize the different behaviour of these tumors during and after treatment when using these new classifications. We believe it indispensable to give a histoprognostic evaluation with each classification.

F. A. LANGLEY, Manchester, England, U. K.:

Of this group of papers I found that of de Brux and Ancla the most interesting. This is probably because much of it is speculative. It seems to me that they have succeeded in reconciling Cohnheim's theory of cell rests

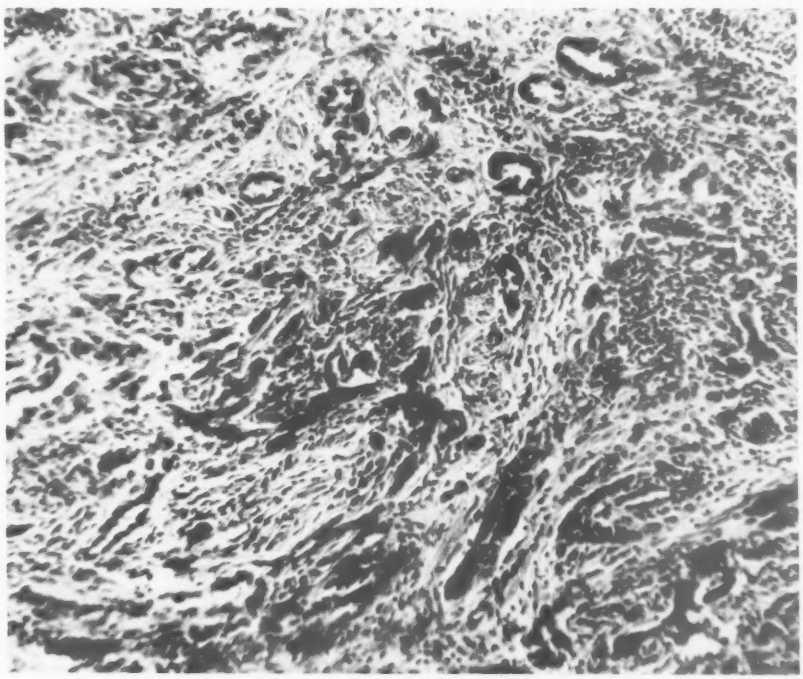


Fig. 6. Small-tubular, partly solid carcinoma located in the lateral cervical wall (remnants of the Gartner's duct could not be depicted).

as the origin of tumors with more recent views based on experimental embryogenesis. I hope my interpretation of their work is correct. While accepting their general approach, it seems to me that 25 tumors is too small a number on which to build such a classification with certainty. This is especially true when one considers how few there must be in some sub-groups. It seems to me that we shall each have to test out this classification with our own material, although the material available to many of us is less than the authors'.

How far do the tumors described as mesonephritic correspond to those of mesonephric origin arising elsewhere in the genital tract? It does not seem to me that the photomicrographs in Figs. 13 and 14 correspond very closely with Teilum's picture of mesonephric tumors, although Fig. 15 may.

### Bibliography

1. Teilum, G.: Acta. path. et microbiol. Scandinav. 34: 431, 1954.

## CLOSING REMARKS

**JEAN A. de BRUX:**

I am very grateful to the discussants for their observations. I am perfectly aware of all the defects of the classification which I proposed, and of the insufficiency of the number of cases. Our conception, which Langley has very well summarized in two lines, is not yet in completely developed form. Once the facts have been integrated into the histogenetic framework, a certain number of tumors of the female genital tract—particularly those of the ovary—will find their proper place, their interpretation and, consequently, perhaps a precise prognosis and indication for therapy. We hope so!

# HISTOCHEMISTRY OF ENDOCERVICAL ADENOCARCINOMA

CONSTANTIN HEROVICI
Villejuif, Seine, France

We have selected fifteen endocervical carcinomas for this study. No lesion is included that had apparent extension to the ectocervix nor any lesion displaying, hysterographically, endometrial involvement.

From a morphological viewpoint these lesions can be divided as follows:

1. True adenocarcinomas
2. Mixed carcinomas (adeno and squamous)
3. Poorly differentiated squamous carcinomas

The techniques used for the histochemical characterization of these carcinomas included the following:

1. Methyl green - pyronin - orange G
2. Feulgen reaction
3. Best's carmine
4. Periodic acid Schiff reaction (PAS)
5. The modification by Marza of the MacCallun technique for demonstration of intracellular potassium.

These techniques have already been described previously in detail in this journal. We could not find any differences between the histochemical characteristics of poorly differentiated squamous carcinomas of the ectocervix

Table I

| Localization of Lesion | Number of Cases | Type I | Type II |
|---|---|---|---|
| Poorly differentiated squamous cell carcinomas of the ectocervix | 40 | 12 | 28 |
| Poorly differentiated squamous cell carcinoma of the endocervix | 7 | 5 | 2 |

Table II

| Histochemical Test for: | Case Number | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Nuclear DNA | +/- | +/- | +/- | +/- | +/- | +/- |
| Nucleolar RNA | + | + | + | + | + | + |
| Cytoplasmic RNA | ++ | + | ++ | + | + | + |
| Glycogen | - | - | - | - | - | - |
| Nuclear Potassium | +/- | +/- | +/- | +/- | +/- | +/- |
| Nucleolar Potassium | + | + | + | + | + | + |
| Cytoplasmic Potassium | ++ | + | + | ++ | ++ | ++ |
| PAS Reaction | + | + | + | + | + | + |

and those of the endocervix. Among these lesions we have found histochemically two profoundly different types. One of them is rich in nuclear DNA, rich in nucleolar and cytoplasmic RNA, rich in nucleolar and cytoplasmic potassium and very poor in glycogen. The other type is characterized by nuclei poor in DNA, but with an increased content of nucleolar RNA, rarely by an increase in cytoplasmic RNA and a negative Best's carmine reaction. (Best's carmine reaction is positive only in very differentiated and mature squamous cell carcinomas.)

Among 7 squamous cell carcinomas, 5 belonged to Type I and 2 to Type II, whereas in a test series of 40 poorly differentiated squamous cell carcinomas of the ectocervix we found 25 of Type II and 15 of Type I.

The adenocarcinomas have a rather constant histochemical pattern and vary only in the concentration of RNA and the cytoplasmic potassium, as shown in Table II. From Table II, we see that the variations of the cytoplasmic RNA behave in parallel fashion with the cytoplasmic potassium.

The 2 remaining mixed carcinomas were from Type II in the squamoid zone and rich in cytoplasmic RNA in the glandular zones. One significant difference between the ectocervical and the endocervical carcinoma is demonstrated by the PAS reaction as well as by the mucicarmin stain. While the PAS reaction is slightly positive and the mucicarmine reaction negative at the ectocervix, we have found 2 squamous cell carcinomas of the endocervix with faintly mucicarminophilic and always PAS positive cells.

Nevertheless, this series of 15 endocervical carcinomas does not permit us to draw any conclusions.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

# CYTOMORPHOLOGY OF THE NORMAL ENDOCERVIX

CARY M. DOUGHERTY
Baton Rouge, Louisiana, U.S.A.

On tissue sections of uterine cervix the typical endocervical cell is the columnar secretory cell. There is but one apparent type. It is tall, it secretes mucinous material, and under certain conditions, not clearly defined, it possesses cilia (1). Correlation of these histologic features is helpful in the interpretation of vaginal smears exhibiting cervical cells. It is the purpose of this study to describe the variations in morphology in endocervical cells as seen on smears. It is more important to be familiar with these variations if the smears are made by scraping the cervix with a spatula, since all manner of cells, as well as small bits of tissue are removed and transferred to the slide in the process.

Smolka (2) identifies two distinct types of endocervical cells, secretory and ciliated, and it is easy to see these types on the smear. However, tissue sections show only a single type cell, which apparently is ciliated in some locations and not ciliated in others. The structure of the cells appears to be the same whether ciliated or not. Because of the ease with which both fixation and mechanical artifacts can be produced in these cells on the smear, one might be cautious in ascribing different qualities to groups of endocervical cells on different specimens. It is possible that there is a cyclic variation in ciliated and non-ciliated cells of the endocervix under hormonal influence. In any event, the columnar ciliated cell will be described as the typical normal endocervical cell.

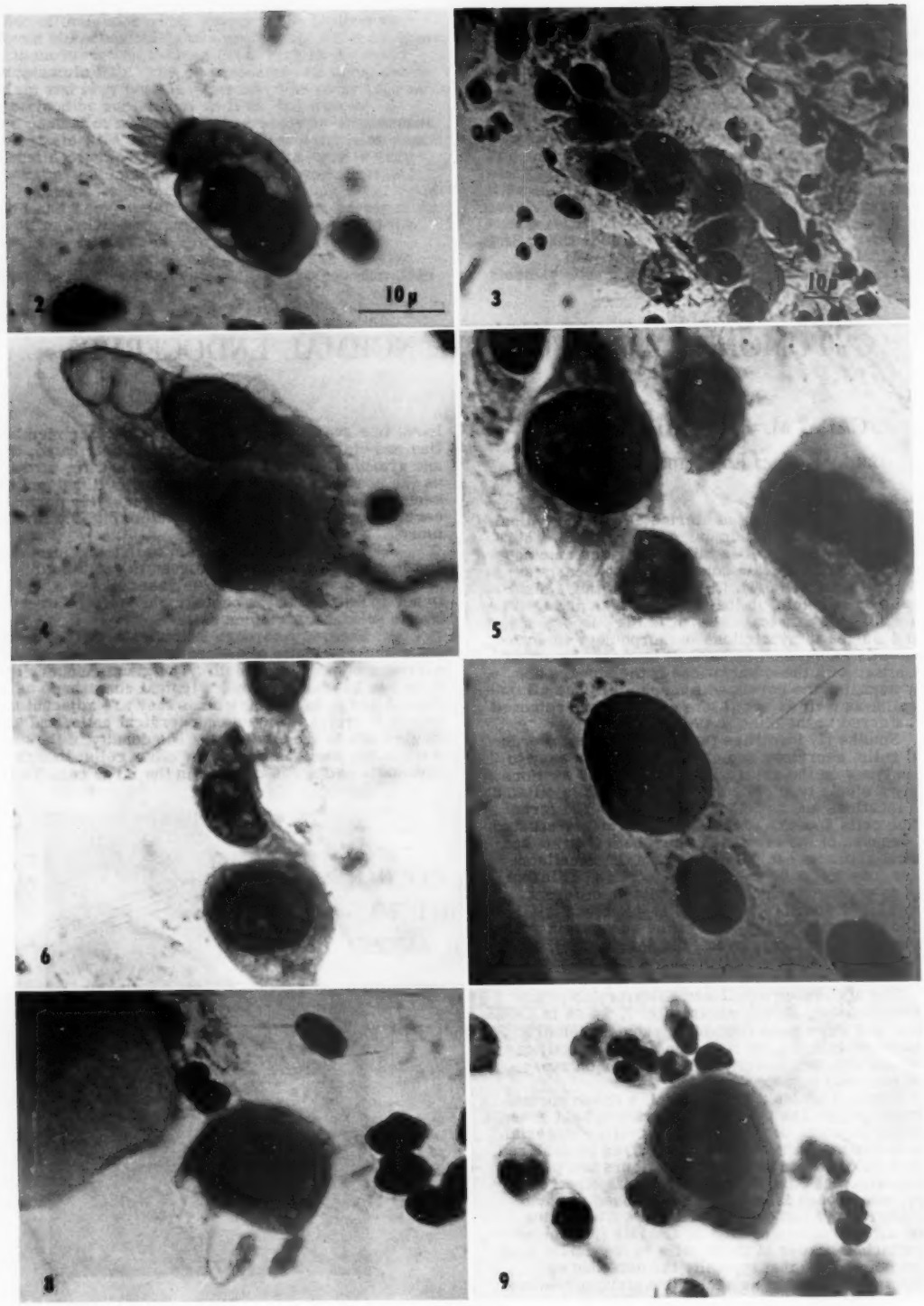
The accompanying illustrations are all of the same magnification, 2800X, except Fig. 3, which is 1300X. Magnifications were measured using photographs of a stage micrometer and each represents total magnification. Additionally, ten-micron marks appear on representative photomicrographs.

Figure 1 shows a group of half a dozen normal endocervical cells. The cytoplasm is stained light green and contains vacuoles or globules of secretory material. There is a distinct distal border stained red or dark red, from which the cilia extend. The cilia fibers are pink. That these are real cilia can be proven with electron microscopy, where each is seen to be composed of some nine peripheral and two central filaments. This is the universal ultrastructure of cilia in animals (3). The proximal or attached border of these cells is frequently cone-shaped or pointed, so that typically the detached cell looks like a small cone. The nuclei are strikingly sharp and regular, and are in groups (Fig. 3); each nucleus looks like every other nucleus. The nuclear membrane is fine and clear, the chromatin evenly distributed, dense and granular. Because of the sharp character of the nucleus the sex chromatin is also sharply outlined and easily seen in these cells (Fig. 3). The completely normal endocervical cell may contain two nuclei (Fig. 2), rarely more.

At times the cytoplasm is thin and almost lacy and in some instances can be seen to contain globules of secretory material (Fig. 4). In cases like this, though it is not so marked in the photograph, there is a resemblance to adenocarcinoma cells.

Figures 5 through 9 illustrate another fairly frequent characteristic of endocervical cells, namely the occurrence of very large cells. The giant endocervical cell often can be identified by its typical cone shape as in Figs. 5 and 6. In these pictures they are adjacent to groups of typical ciliated endocervical cells, and several single cells of the same type. The density of the nuclear stain is the same as that of the other cells, though the chromatin pattern is coarser in the large cell. To this

Fig. 1.




Figs. 2-9.

cell we might appropriately apply Ruth Graham's favorite admonition to students, "There's nothing wrong with that cell except its large size!"

Figure 7 depicts three isolated nuclei. Taken by themselves, it may not be possible to identify these cells. However, on this slide they were found in a group of endocervical cells, both ciliated and non-ciliated. In this context these nuclei could be identified. It will be noted that the nuclei of Fig. 7 are similar to those of Fig. 5, and, in fact, both are from the same patient.

Figures 8 and 9 show cells which are interpreted as large endocervical cells with distortion of the cytoplasm. Again, in both instances there were normal endocervical cells in the immediate vicinity on the slide. While this point is not put forth as being conclusive, one is justified in judging a cell by the company it keeps.

In summary then, one may say that the columnar secretory cell is the typical cell of the endocervix. The basic architecture of the cell is sometimes altered by more than one nucleus, by the growth to an unusually large size, by the fragility of the cytoplasm, its distortion or its disappearance altogether, and by an unusually dark nuclear stain. It is mainly identified by its uniformity, its occurrence in groups, or by the association of the cell in question with other typical columnar endocervical cells.

Bibliography


1. Papanicolaou, G. N.: Atlas of Exfoliative Cytology. Cambridge, Mass., 1954, Harvard.
2. Smolka, H.: Acta Cytologica 2:515, 1958.
3. Fawcett, D. W. and Porter, K. R.: J. Morphol. 94: 221, 1954.


## DISCUSSION

MARIA S. BLANCO de del CAMPO, Palo Alto, California, U. S. A.:

I agree that cyclic changes occur in endocervical epithelium. Although I question Dougherty's decision of selecting the ciliated cell as the typical normal endocervical cell, I have observed a "ciliated crown" suggesting that some non-ciliated cells in smears have actually lost cilia.

CLARICE AMARAL FERREIRA, Rio de Janeiro, Brazil:

At the beginning of the proliferative phase the columnar cells of the endocervix are low, with basally located and well stained nuclei; the stroma is dense and fibrous. From the eighth to the fourteenth day, the columnar cells grow higher, becoming taller and broader, with centrally located nuclei. The distal part of the glandular cells bends because of the mucus secretion (the "protrusion of the secretory cells" of Papanicolaou). The stroma is edematous. Near ovulation, dense, round cells, with abundant mucus, and often with a half-moon shaped nucleus pushed to the border (signet-ring cell) can be seen. Also one may find free nuclei. After ovulation the columnar cells shorten progressively, the mucus secretion diminishes and the stroma becomes dense again. At the end of the luteal phase the tall columnar cells with basally located nuclei can once more be encountered.

Bibliography


1. Ernest, A. and Guzman, F.: El Citodiagnostico en Ginecologia. VI º Congreso Chileno de Obst. y Gineco 1955.


EMMERICH von HAAM, Columbus, Ohio, U. S. A.:

We observe small non-secreting cells with distinct cuticles and large cells filled with mucus vacuoles; occasionally the small cervical cells appear ciliated. I do not believe that secretory cells may become ciliated after having excreted their mucus. I consider them as two distinct cytological entities.

## CLOSING REMARKS

CARY M. DOUGHERTY:

For a better understanding of cell types seen in smears it seems best to go back to sections of tissue from which they come. Figure 1 is a mosaic of high pow-

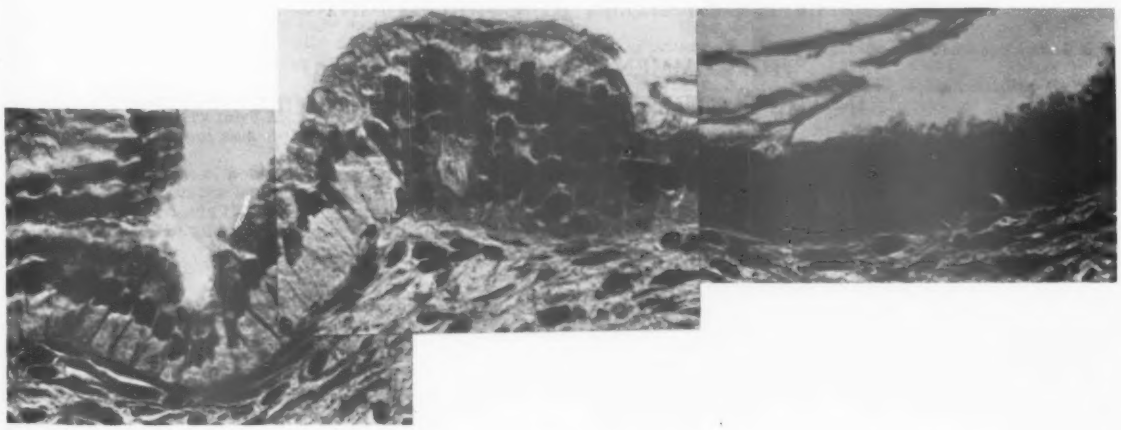


Fig. 1.

er micrographs showing the cervical epithelium of a single gland, with glandular cells exhibiting two different forms. On the left, secretory type cells are seen with subnuclear globules which push the nuclei more than half way up. The peripheral portion of these cells does not contain secretory material, and in this respect they resemble cells of the second type. Those cells on the right are of the ciliated variety. A junction or transition of the two phases of cell morphology is seen in the mid-portion of the illustration. Here, some cells appear to be changing from the ciliated to the secretory phase. In other places in this same specimen cells were noted which contained both secretory globules and cilia, the globules appearing either proximally or distally to the nucleus.

Unless this change is observed in tissue culture of living cells, it is, of course, not possible to say that a certain progression of events takes place. To me, the evidence that the same cervical cells have at least two different phases is summed up by the observations that 1. ciliated and secretory cells are found in the same specimen arrayed in long rows of one or the other form, rather than alternately placed in small groups as in the tube, 2. ciliated cells containing globules of mucinous material aggregated either beneath or distal to the nucleus have been repeatedly observed in tissues, and 3. secretory cells possessing cilia also have been noted.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

# CYTOCHEMISTRY OF NORMAL AND ABNORMAL ENDOCERVIX

CONSTANTIN HEROVICI
Villejuif, Seine, France

The histological material for this study was furnished by 10 surgical specimens of hysterectomies performed for myomatous uteri in which the endocervix is perfectly normal, as well as by 40 endocervical biopsies which showed polypoid endocervicitis with and without squamous metaplasia.

We have employed our histochemical routine which includes:

1. Methyl green—pyronine—orange G
2. The Feulgen reaction
3. Best's carmine
4. PAS reaction
5. A variant of the Marza technique by MacCallum for demonstration of cellular potassium.
6. Alcian blue and mucicarmine

### NORMAL ENDOCERVICAL EPITHELIUM

This cylindrical epithelium is characterized by cells with nuclei which contain only small amounts of DNA. The cytoplasm of these cells contain rather marked concentrations of RNA, which is also found in the nucleoli.

Glycogen is not found in normal endocervical cells. On the contrary the PAS reaction is positive in the cells of the epithelium and in the cells of the glandular tubules.

The amount of potassium is very small in the nuclei, but higher in the nucleoli and the cytoplasm. Here as elsewhere one finds the same parallelism between the distribution of RNA and intracellular potassium.

### POLYPOID CERVICITIS

Without being able to attribute a precise characteristic to this inflammatory lesion we have consistently found three histochemical differences as compared to the normal endocervix:

1. A pronounced elevation of RNA in cytoplasm and nucleoli.
2. Elevation of cytoplasmic potassium.
3. Generally a positive PAS reaction.

In the squamous metaplastic zones these modifications are more pronounced. Moreover one finds cells of endocervical epithelium in which the PAS reaction, the alcian blue stain and the mucicarmine reaction reflects a high degree of maturity.

## DISCUSSION

ALVAN G. FORAKER, Jacksonville, Florida, U. S. A.:

There is little comment to make on Herovici's interesting notes. Leuchtenberger (1) found DNA-containing "cytoplasmic inclusions" in 63 human rectal polyps. Perhaps this search could be advantageously extended to "polypoid cervicitis" and endocervical polyps. It should be noted that endocervical cells are usually PAS positive. This is not due to glycogen, but to mucin, and resists malt diastase digestion (2). Endocervical cells give a strong cytoplasmic tetrazolium reaction for reductases (3). Inflammatory cells in cervicitis (3) also show evidence of reductase content.

Bibliography

1. Leuchtenberger, C.: Laboratory Investigation 3:132, 1954.
2. Foraker, A. G. and Marion, G.: Am. J. Obst. & Gyn. 72:400, 1956.
3. Foraker, A. G., Denham, S. W. and Aguilar Celi, P.: Cancer 7:311, 1954.

## CLOSING REMARKS

CONSTANTIN HEROVICI:

May I thank Dr. Foraker for his judicious remarks. May I point out that I have, in effect, found cytoplasmic inclusions with DNA in endocervical carcinoma after irradiation with radium. As I have noted, the endocervical cells were PAS positive. I did not think at all of mucin (acid mucopolysaccaride). Since, according to Mowry I have proceeded with the PAS technique using Alcian Blue and in a very short period I completed it once with Aurantia. When I say that one cannot see glycogen in the cells I think of the results with Best's Carmine and Lugol's test as to the reaction of tetrazolium in regard to the reduction. I have not used it in my work.

# FLUORESCENCE MICROSCOPY OF CERVICAL CELLS AND MACROPHAGES

LUDWIG von BERTALANFFY*
Topeka, Kansas, U.S.A.,
and
FELIX D. BERTALANFFY†
Winnipeg, Canada

The fluorescence microscope method of cytodiagnosis, which was recently developed (1,2,3), the procedure of which is described elsewhere (2,4), is an excellent tool to ascertain the activity of the endocervical epithelium.

Normally cells from the endocervical epithelium, which is composed of columnar ciliated and secretory cells, show reddish-brown cytoplasmic and green or yellow nuclear fluorescence. The ciliated cells have a somewhat denser cytoplasm, the nuclei are normally present in the middle portion of the cells, and reddish-brown cilia are often discernible. The secretory cells have a finely reticulated cytoplasm which shows similar reddish-brown fluorescence, and the nuclei are usually present near the basal portion of the cells. Ciliated and secretory cells cannot be distinguished readily when endocervical cells are only seen en face in "honeycomb formation."

Cases of endocervical erosion usually show so-called "active" endocervical cells with increased red cytoplasmic fluorescence. The reason for this seems to be that these cells, presumably having a higher than normal rate of proliferation, contain larger amounts of RNA than normal endocervical cells. However, the endocervical cells of erosions usually show normal morphology (or only slightly enlarged nuclei), and therefore are readily distinguished from suspicious cells.

"Active" endocervical cells are also occasionally encountered in otherwise normal smears. In most cases they are present only temporarily. A logical reason for the temporary presence of "active" endocervical cells would be that at times, under endocrine stimulation, the endocervical epithelium may proliferate at a somewhat higher rate than usual. The cells then contain increased amounts of RNA, and consequently show more intense reddish cytoplasmic fluorescence.

These fluctuations of endocervical cell proliferation could be in line with those of the vaginal epithelium whose thickness varies with the stages of the menstrual and estrous cycles.

Further follow-up studies have to ascertain whether or not persistent "active" endocervical cells ever give rise to actual atypical lesions of the cervix.

Macrophages in cervical and vaginal samples normally show brownish fluorescent cytoplasm and green to yellow nuclei. In cases of chronic inflammations, e.g., cervicitis, the cytoplasm of macrophages may be reddish brown, presumably owing to a higher RNA content associated with an increased rate of proliferation brought about by chronic irritation. In such cases often larger numbers of macrophages are present.

Bibliography

1. Bertalanffy, L. von, F. Masin, and M. Masin. Sci. 123:1024, 1956.
2. Bertalanffy, L. von, M. Masin, and F. Masin. Cancer 11:873, 1958.
3. Bertalanffy, L. von, and F. D. Bertalanffy. What's New (Abbott Laboratories) No. 214, Early Winter Issue, 1959.
4. Bertalanffy, L. von. Klin. Wschr. 37:469, 1959.

*Menninger Foundation, Topeka, Kansas, U. S. A.
†Department of Anatomy, Medical Faculty, University of Manitoba, Winnipeg, Canada. This work is supported by a research grant from the National Cancer Institute of Canada.

# DIFFERENTIATION OF ENDOCERVICAL GLANDULAR AND METAPLASTIC CELLS

JEAN A. de BRUX AND
J. DUPRÉ-FROMENT
Paris, France

The differentiation between the endocervical cell and the so-called "metaplastic" cell presents no difficulty, for these are two cellular types clearly distinct and easily recognizable.

The one is columnar and arises either in the border mucosa or in the epithelium of the endocervical glands. The other is of epidermoid aspect, for it is exfoliated from a metaplastic epithelium, hence, its designation as a "parabasal" cell (Fig. 1).

In fact, the reserve cells situated at the foot of the endocervical columnar elements have multiplied to such an extent as to form, in their stead, an incomplete epithelium consisting of several layers of cells, all of basal type; later, this may ripen and become differentiated into deep, intermediate and superficial layers. Hence, a finding on the smear of the presence of "parabasal" elements indicates that one is dealing with a metaplastic epithelium still young and relatively unorganized.

The endocervical cell is an element of columnar form with an eccentric nucleus. The rather poorly outlined cytoplasm is blue-grey, either homogeneous and

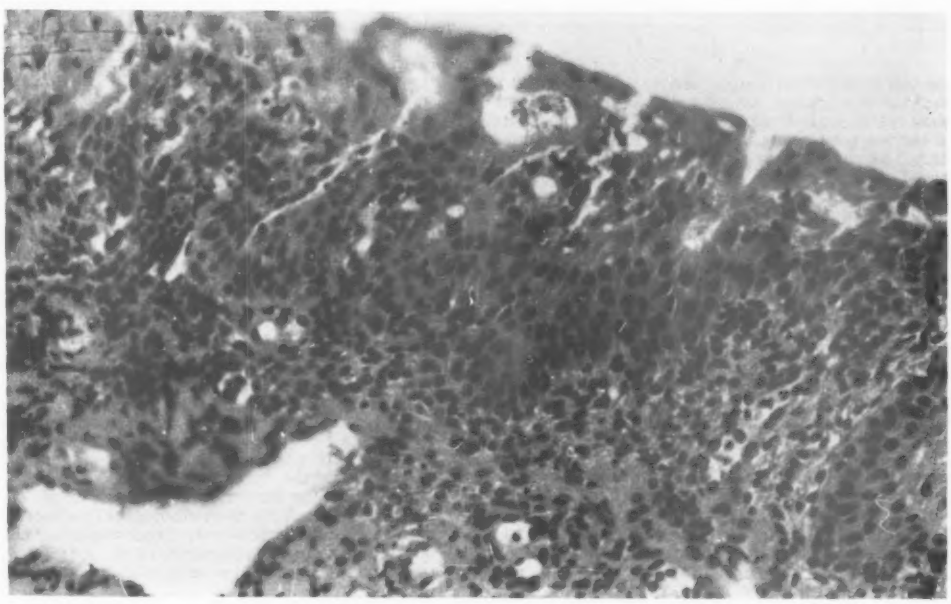


Fig. 1. Metaplasia.

| | HYPERPLASTIC ENDOCERVICAL CELL | ENDOCERVICAL CELL IN EOSINOPHILIC METAMORPHOSIS |
|---|---|---|
| Form | Columnar | Racquet-form |
| Grouping | Isolated<br>In fringes<br>In clumps (heap of marbles)<br>In wide shreds of endocervical mucosa<br>Naked nuclei | In stretched shreds consisting of contiguous cells with vague cytoplasmic outlines |
| Size | 15-20 $\mu$ | Slightly larger than 15-20 $\mu$ |
| Cytoplasm | Moderately cyanophilic | Polychromatophilic |
| Nucleus | Rounded<br>Situated at one extremity of the cell<br>Regular<br>Bluish<br>Isonucleosis | Rounded<br>Situated at the rounded extremity of the cell, or approaching its center<br>Regular but prominent<br>Violet-tinged<br>Isonucleosis |
| Nuclear-Cytoplasmic Ratio | Normal | Normal |
| Chromatin | Finely specked<br>Nuclear membrane slightly accentuated<br>Nucleolus | Finely specked<br>Nuclear membrane slightly accentuated<br>Nucleolus |

| "PARABASAL" CELL OR METAPLASTIC CELL | CELL OF ACTIVE, UNDIFFERENTIATED AND IMMATURE METAPLASIA, OR OF HYPERPLASIA, OF THE RESERVE CELLS |
|---|---|
| Oval, with stretched cytoplasm<br>Rounded, of basal type<br>Navicular, junction-cell type | Oval, with stretched cytoplasm |
| In clumps or patches, with vague cytoplasmic outlines<br>In bands of distinctly-outlined cells disposed in a single line | In clumps or, more often, in stretched shreds consisting of contiguous, vaguely-outlined cells |
| Equal to or larger than a basal cell | Slightly larger than a columnar cell |
| Polychromatophilic or frankly cyanophilic—sometimes vacuolized | Cyanophilic |
| Rounded or oval<br>Central<br>Regular<br>Violet-tinged or cyanophilic<br>Isonucleosis | Rounded or oval<br>Central<br>Regular but prominent and "obese," sometimes distended, sometimes with dyskaryotic tendency<br>Cyanophilic or clear<br>Slight anisonucleosis |
| Normal | Normal or slightly inversed |
| Finely and densely specked<br>Nuclear membrane slightly accentuated<br>Nucleolus visible or invisible | Specks accentuated, grainy, or chromatin sparse, vacuolized, with grains few in number and evenly distributed<br>Nuclear membrane heavily outlined<br>Vacuole prominent |

dense, or microvacuolar; more rarely, it is occupied by one vacuole which rounds out the cell, and compresses and pushes the nucleus toward the periphery. The nucleus is round and its membrane is finely outlined and perfectly regular. The chromatin is finely speckled, more or less striped, and most often shows a small, clearly visible nucleolus.

The cells appear disposed in fringes or in masses, or may form shreds of mucosa. At the level of the fringes, their columnar character is clearly evident and, in some cases, after touching the cervix with acetic acid, one may even discern a ciliated border.

Where the cells appear in masses or in patches, the cytoplasmic limits become effaced and the ensemble appears monomorphous, consisting of nuclei side by side, all of equal size (Fig. 2).

The parabasal or metaplastic cell is larger, with oval or rounded cytoplasm, homogeneous or sometimes vacuolized. In some cases, the cell is almost navicular. It has a characteristic tinctural affinity: either violet-tinged and polychromatophilic, or, when the epithelium of its origin is more organized, very cyanophilic. Moreover, in the first case, the cells are grouped in masses or in stretched shreds in which the cells are contiguous; in the second case, the cells are more likely to appear well separated and often disposed in bands (Figs. 3,4).

Their nuclei are central, very regular, round, of hyperplastic aspect, and prominent. They may be violet-tinged or cyanophilic, and their chromatin speckles dense, although fine. The nucleoli are rarely visible.

For this description to be quite complete, however, there should be added a third cellular type, which appears to fall just between the two preceding ones. This is what one might call the endocervical cell "transformed," or in process of eosinophilic metamorphosis. This phenomenon, like that of metaplasia, appears as the result of a state of cellular suffering, particularly when the subjacent stroma is the site of an extensive inflammation, and would even seem to constitute the precursor state of metaplasia. It is found rather frequently at the border of ectropions and polyps. These columnar cells then protrude, and their swollen, rounded extremity lends to them a characteristic racquet-like appearance. Their cytoplasm becomes eosinophilic and finely granular, and their nuclei, more markedly violet-tinged, move toward the center of the cell (Fig. 5).

On the smear, the aspect of these cells is somewhat different from the pathological picture: their eosinophilia changes into a polychromatophilia, but their recquet-form remains characteristic, and their grouping is in shreds of contiguous cells whose edges often appear fuzzy (Fig. 6).

Actually, therefore, three sorts of elements may be distinguished on the cervical smears:

a. the endocervical cell, whose nucleus may or may not be hyperplastic;
b. the endocervical cell in metamorphosis, polychromatrophilic and racquet-formed;
c. the "parabasal" cell of metaplasia, polychromatophilic or cyanophilic, with hyperplastic nucleus.

The presence of the first type in abnormal quantity signifies the existence of an endocervical hyperplasia, an ectropion or a polyp. In a later subject we shall study how to diagnose each of these lesions.

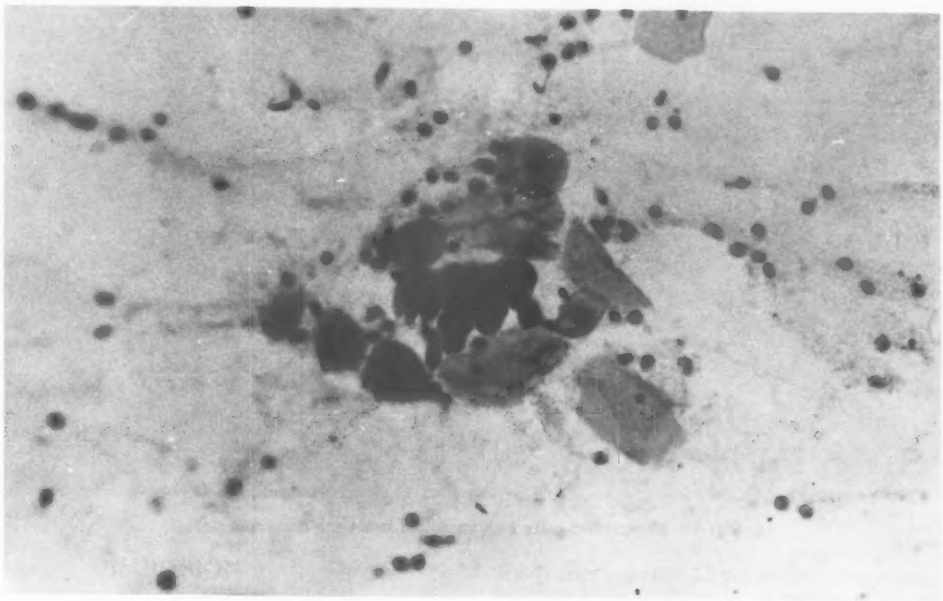


Fig. 2. Hyperplasic endocervical cells.

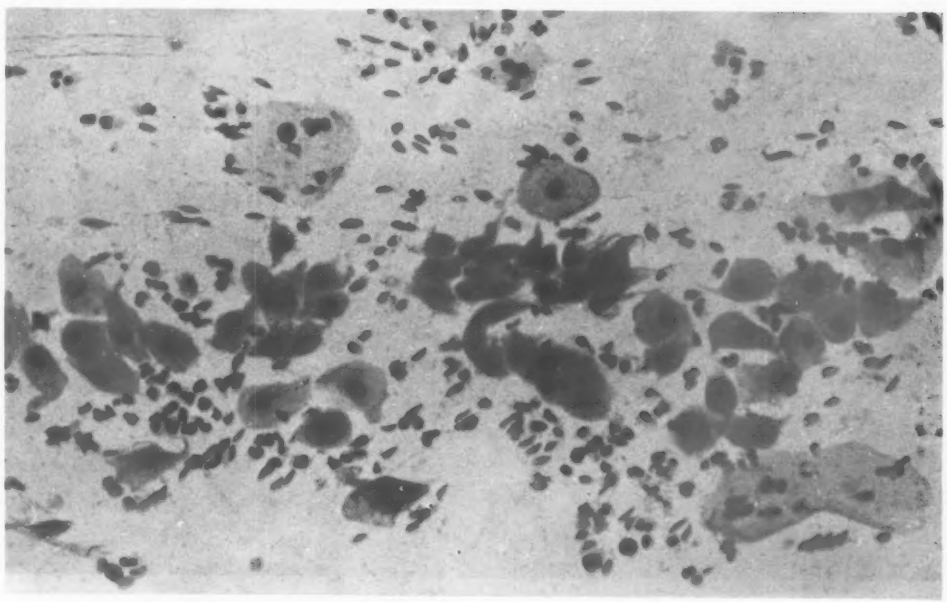


Fig. 3. In the middle: endocervical cells in metamorphosis. On each side: metaplastic cells of parabasal type.

Fig. 4. Metaplastic cells with vacuoles; some are macrophagic.

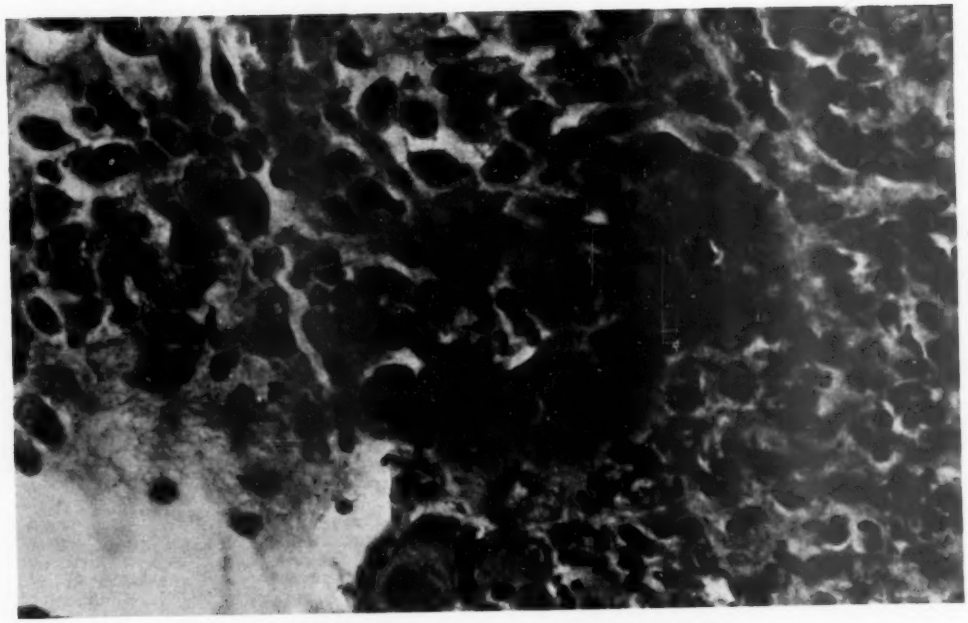


Fig. 5. Exfoliation of metamorphosed endocervical cells.

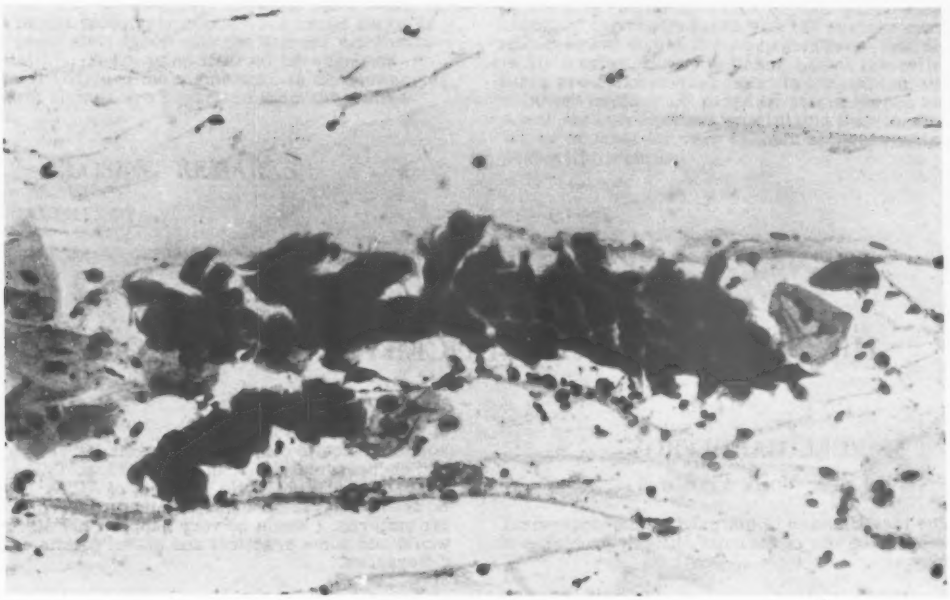


Fig. 6. Metamorphosed endocervical cells.

## DISCUSSION

RAIMUND KRIMMENAU, Dresden, Germany:

Well-preserved columnar cells of the endocervix are easily discerned from differentiated squamous epithelia. One finds such well-preserved cells in varying amounts according to the techniques employed in their preparation. Scraping with a wooden spatula according to Ayre shows far more such cells than does a preparation technique which does not damage the epithelial surface, for example, the use of a cotton applicator. By the latter technique one often finds degenerated, stripped nuclear elements sometimes with enlarged nuclei, which can be confused with tumor cells if they occur in dense clusters.

Histologically, metaplastic lesions shows small cells with a thin rim of cytoplasm below the cylindrical epithelium, which sometimes only partially covers these cells. Parabasal cells, frequently darkly colored, arranged like a string of beads, or sometimes lying in individual clusters with a non-atrophic cell pattern are proof of a metaplastic process. With the increasing differentiation towards the squamous epithelium, the cells appear more similar to intermediate cells.

Cylindrical cells on the surface of the ectocervix (with ectopy) with or without subepithelial inflammation often show a partial cubic conversion. Frequently nuclear and cellular edema is found. Such cells, torn from the cellular formation, are then in conformity with Figures 3, 5 and 6 of the above authors. The term "metamorphosed" appears to be very useful. Mucus stainings are usually positive. As the above authors mentioned, the tendency towards dyskaryosis exists, which raises the question of the differential diagnosis from tumor cells.

ALEXANDER MEISELS, México, D. F., México:

It is evident that the differentiation between endocervical glandular and metaplastic cells is easy. What is more difficult is the correct recognition of the metaplastic cells as such. In many cases, there is little trouble, because the smears contain almost only superficial squamous cells and groups of typical endocervical cells. In these cases, the bands of "parabasal" cells are easily recognized as being of metaplastic origin. The tinctorial affinity of these cells also makes them stand out, but most characteristic is their appearance in bands or shreds, with touching cellular membranes, but without overlapping. When typical, these cells are quite easily diagnosed. When, however, the smears contain abundant parabasal cells, the differentiation between these and the metaplastic cells may be very difficult and require the administration of very small doses of estrogens, which will change the ectocervical cells into superficial elements, leaving the metaplastic cells standing out as the only remaining "parabasal" cells. Another difficulty is the recognition of superficial metaplastic cells from the superficial ectocervical cells. Again the grouping and tinctorial affinity should help.

## NO CLOSING REMARKS

# HISTIOCYTES AND ENDOCERVICAL CYTOLOGY

MARCEL GAUDEFROY
Lillie, Nord, France

The identification of histiocytes in endocervical smears seems to be one of the most difficult problems of colpocytology.

1. Multinucleated giant cells (described in Figure 23, p. 134, of Vol. II, No. 1, 1958, of ACTA CYTOLOGICA) are certainly histiocytes. It is possible to find such giant cells in the cystic endocerfical glands (Nabothian cysts) in histological sections.
2. Another type of histiocyte is the large and giant phagocytic cells, with several vacuoles in which ingested leukocytes or cellular debris are observed; the nucleus is usually eccentrically located.
3. The well-preserved, normal histiocyte has a finely vacuolated, foamy, pale, cyanophilic, and indistinctly outlined cytoplasm and an eccentric, oval or bean-shaped nucleus often with marked nucleoli. In groups, these histiocytes are near each other, neither overlapping nor in large clusters, but often in streaks.
4. The well-preserved endocervical cells are rather easy to recognize. They are almost always in large sheets with smoothly granular, frequently overlapping nuclei; very marked nucleoli are not encountered, nor is the cytoplasm clearly outlined. The size of the nuclei are variable but the shape remains round or slightly oval. The nuclei are not in the center of the cell but are not touching the cellular membrane either. In numerous cases, it is possible to see the cilia of columnar cells and their conical shapes.
5. The really difficult problem is the differential diagnosis between histiocytes and endocervical cells exhibiting degenerative changes. One might say that histiocytes vary in size, remaining round or oval. In general, the size of histiocytes is larger than the size of endocervical cells. The nucleus of the histiocytes is clearer, with finely granular chromatin and a transparent appearance.

In the above description I have tried to describe some diagnostic differential aspects between histiocytes and endocervical cells. In Figure 22, p. 130, and Figure 24, p. 137, of Vol. II, No. 1, 1958, of ACTA CYTOLOGICA no definite agreement was obtained concerning the cellular patterns. I would be very happy if the discussants would add some practical and useful details to clarify the differences.

## DISCUSSION

MARIA S. BLANCO DE DEL CAMPO, Palo Alto, California, U. S. A.:

These are useful points to follow in distinguishing histiocytes and endocervical cells. However, my observations differ from Gaudefroy in that I have seen nucleoli as a common finding in well-preserved endocervical cells, and in severe cervicitis these cells may have two or more nucleoli which often are so markedly enlarged as to cause misinterpretation. In addition, I think it is worth pointing out that the nuclei fill almost the whole endocervical cell when these are seen in their characteristic honeycomb grouping. Also poorly preserved endocervical-cell clusters quite often appear as a syncytium of nuclei in an amorphous cytoplasm that may be easily mistaken for giant histiocytes.

EMMERICH VON HAAM, Columbus, Ohio, U. S. A.:

My most important criterion of differentiating between endocervical cells and histiocytes is the fact that the cytoplasm of endocervical cells is usually less in amount than that of the histiocytes, unless it is filled with easily recognizable mucous droplets. In my opinion, the cells in the picture to which Gaudefroy refers are all endocervical cells.

ALEXANDER MEISELS, Mexico, D. F., Mexico:

In most cases there is no difficulty in distinguishing between histiocytes and endocervical cells. Their grouping and appearance are characteristic. The problem arises when both occur in atypical groupings, with scarce cytoplasm, or when only isolated naked nuclei are present.

In these cases, a careful examination of the smears may discover some typical histiocytes or endocervical cells. If this is not the case, one may find one or a few nuclei with a small indentation in the membrane, or even a nucleus with the typical kidney shape, which

will brand the cells as histiocytes. On the other hand, endocervical nuclei frequently contain one small clump of chromatin completely fused with the nuclear membrane (sex chromatin?). There is no interval between this clump of chromatin and the membrane. In the histiocytes, the chromatin clumps are separated from the nuclear membrane.

## CLOSING REMARKS

MARCEL GAUDEFROY:

I thank the discussants and I am very interested by the differential criterion pointed out by Meisels: small clump of chromatin fused with the nuclear membrane in endocervical nuclei and separated from that membrane in histiocytes. Like von Haam, I think the cells in the pictures are endocervical cells. In my opinion, the markedly enlarged nucleoli observed by Maria Bianco and more a test for endocervical hyperplasia than for endocervicitis, or at least for very chronic endocervicitis with hyperplastic reaction.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

# CYTOLOGY OF ENDOCERVICITIS

EMMERICH von HAAM
Columbus, Ohio, U.S.A.

Endocervicitis, either acute or chronic, is one of the most frequently encountered gynecological conditions, and one may safely state that it can be found in five out of seven cervices examined. It must be differentiated from cervical ectopy, cervical erosion and cystic cervicitis, although combined lesions are common. The classic endocervicitis consists of an inflammatory process limited to the submocosa of the endocervix which does not extend beyond the squamo-columnar junction of the external os. For this reason it does not produce any visible lesion. Endocervicitis is usually not associated with the formation of cystic cervical glands, but in its chronic form leads to the development of an endocervical granulation tissue with subsequent fibrosis, scarring and stenosis of the endocervical canal. It always produces an exudate, which in acute cases is purulent in nature while in chronic cases it contains many macrophages. Endocervical cells exfoliate in large numbers and usually show evidence of cellular injury and degeneration. This is in contrast to cervical erosion, where cervical cells are often excellently preserved. The three types of endocervical cells mentioned by Frost (1) usually can no longer be distinguished, since the cilia with the terminal bar have disappeared and the cytoplasm has undergone various forms of degeneration (2). Sometimes the degenerative process is so advanced that only large endocervical nuclei remain which may be of concern to the cytopathologist (3). Pseudoeosinophilia, which is so often observed in inflammatory processes of the portio vaginalis, is rarely present in endocervicitis. The amount of mucus is increased but not excessive. Metaplastic squamous cells are particularly common when the endocervicitis is combined with cervical erosion (4), but even without involvement of the external os, epidermization of the endocervix may be extensive and can be explained by the stimulus of infection (5). The brownish discharge of some cases of endocervicitis can be explained by the admixture of red blood cells extending from the granulation tissue, and sometimes the endocervical canal is so friable that bleeding follows even the most gentle technique of obtaining a smear (6).

To us the cytological diagnosis of endocervicitis has never been difficult, and we have never been much troubled by the recognition of mature or immature metaplastic epithelial cells. We also have rarely been troubled with epithelial dysplasia, which is so frequent in inflammatory changes of the ectocervix.

Bacteriological evaluation of endocervicitis, acute or chronic, is difficult and has never been too satisfactory in our hands. The gonococcus, which is the typical causative agent of this lesion, seems to have disappeared from the flora of inflamed endocervices and has been replaced by the usual pyogenic cocci. Trichomonas are rare, and the question if Trichomonas can cause endocervicitis still needs further clarification (7).

Bibliography

1. Frost, J. K.: Gynecologic and Obstetric Pathology (E. Novak and E. R. Novak), Philadelphia, 1958, W. B. Saunders Co.
2. Graham, R.: The Cytologic Diagnosis of Cancer. Philadelphia, 1954, W. B. Saunders Co.
3. Frost, J. K.: Gynecologic and Obstetric Pathology (E. Novak and E. R. Novak), Philadelphia, 1958, W. B. Saunders Co.
4. Streicher, H. J. and Sandkuhler, S.: Klinische Zytologie. Stuttgart, 1953, Georg Thieme.
5. Fluhmann, C. F.: Am. J. Obst. and Gynec. 68:1447, 1954.
6. Smolka, H. and Soost, H. J.: Grundriss und Atlas her gynakologischen Cytodiagnostik. Stuttgart, 1956, Georg Thieme.
7. Trussell, R. E.: Trichomonas vaginalis and Trichomoniasis. Springfield, Ill., 1947, Charles C. Thomas.

MARCEL GAUDEFROY
Lille, Nord, France

The cytological patterns of chronic endocervicitis are different in vaginal and cervical smears because of the way in which the cytological material is obtained.

1. The vaginal smear is obtained, in most cases, by pipette aspiration or by spatula after introduction of a speculum. The smear is composed mainly of vaginal cells, more or less well preserved, and with various inflammatory changes present. The most interesting characteristics are the presence of purulent mucus in irregularly colored streaks, often eosinophilic. Endocervical cells, polymorphonuclear leuko-

cytes and histiocytes are seen in the mucus streaks. The endocervical cells are almost always degenerated with cytoplasmic debris, or without any cytoplasm, and frequently there is karyorrhexis, irregularity of nuclear size but not of shape, and conservation of a smoothly granular chromatin. The polymorphonuclear leukocytes are often crushed, sometimes scattered and mostly degenerated.

2. The endocervical smear is almost always prepared after the endocervical mucus has been wiped away. The endocervical smear is therefore "cleaner" than the vaginal smear and without purulent mucoid streaks. Polymorphonuclear leukocytes are scarce. Endocervical cells are isolated or in small, sometimes overlapping, clusters. The nuclei are often naked, irregular in size, anisokaryotic, and with some small nucleoli and a fine chromatin structure. The large nuclei appear swollen. Histiocytes are sometimes present and their nuclei are difficult to differentiate from isolated endocervical nuclei.

RAIMUND KRIMMENAU

Dresden, Germany

Inflammatory processes in the distal cervical canal with spreading to the surface of the ectocervix (mostly with simultaneously existing ectopy) are extremely frequent. Clinically there exists a stronger tendency toward contact bleeding. Colposcopically exposed vessels of the connective tissue at erosio-vera areas are seen which in certain cases may cause difficulties in the differential diagnosis. Histologically (conization is excellent therapy for such persistent changes, whereby we obtained incontestable material of examination) leukoplasmocytic infiltrations are found, which, however, only rarely also reach the proximal portion of the cervix, expanded epithelial exposures beside regenerative epithelium of increased proliferation. Metaplastic indifferent cellular portions partially exhibit a very "unquiet" appearance due to inflammatory infiltration and irritation. In addition, there is inflammatory swelling of the stroma cells and capillary endothelium. However, epithelial changes of marked degree with hyperchromatic polymorphous nuclear patterns are very rare compared to the frequency of this entity.

The knowledge of the histological changes is the foundation for the understanding of the cytology of endocervicitis. In most cases, contrary to the clinically alarming appearance of the ectocervix, around the external os there are found, cytologically, only "simple" inflammatory patterns even after repeated examinations: abundant leukocytes and histiocytes, very often trichomonads, and cellular epithelial cells of the intermediate and parabasal layers. The borders are often blurred due to bacteria or also due to overlying leukocytes and autolysis. Parabasal cells with vacuolizations and frequently with eccentric nuclei are often found with epidermizations without any pronounced inflammation. Wied very suitably called them "metaplasia cells" (3). Likewise, degenerative cervical cells are not alone characteristic for inflammation, although they are especially impressive in this case as free, partly swollen nuclei. Thus, in most cases, relatively easy cytological diagnosis as "unsuspected" on repeated smears is the best differential diagnostic procedure on the "bleeding ectocervix."

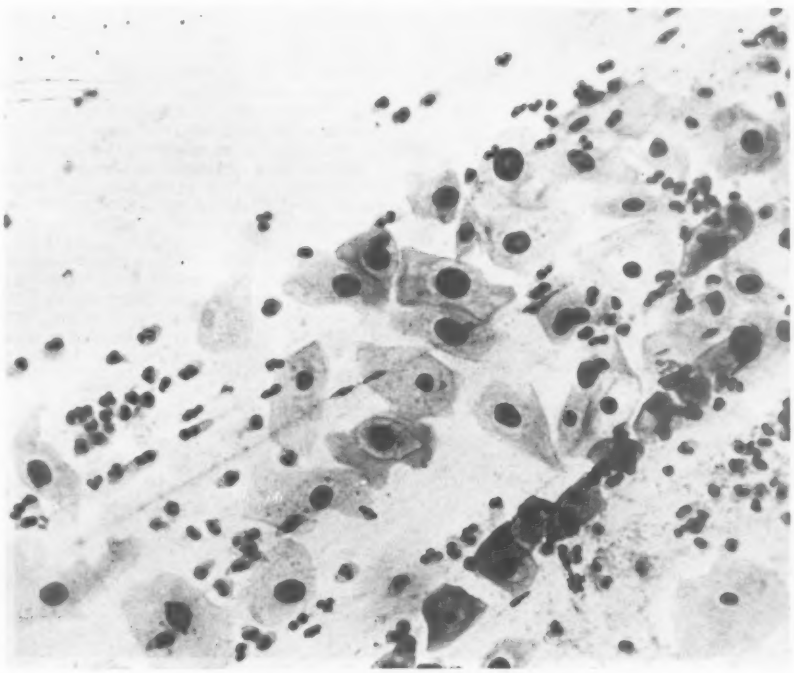


Fig. 1. Dyskaryosis with halo-formation.

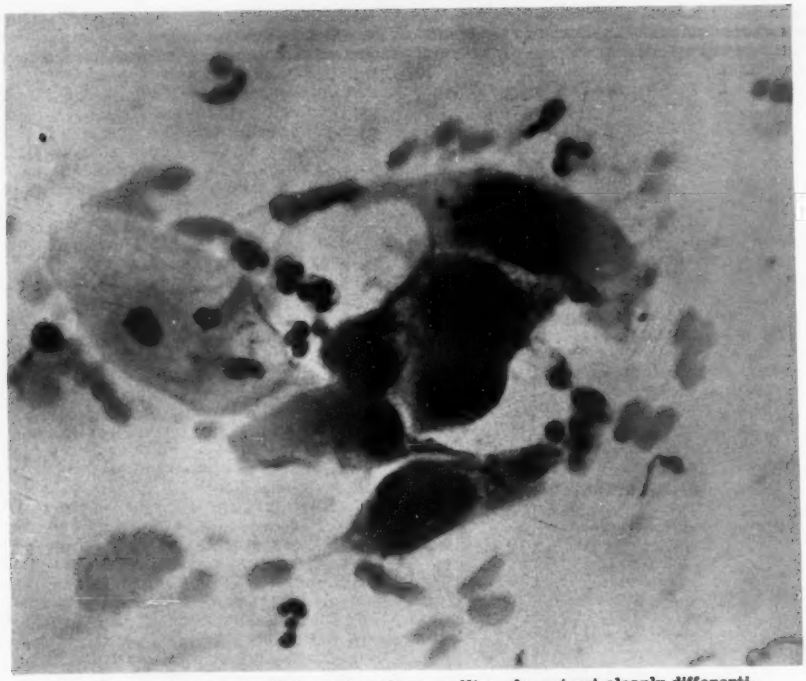


Fig. 2. Degenerative cellular and nuclear swelling of a not yet clearly differentiated epithelium.

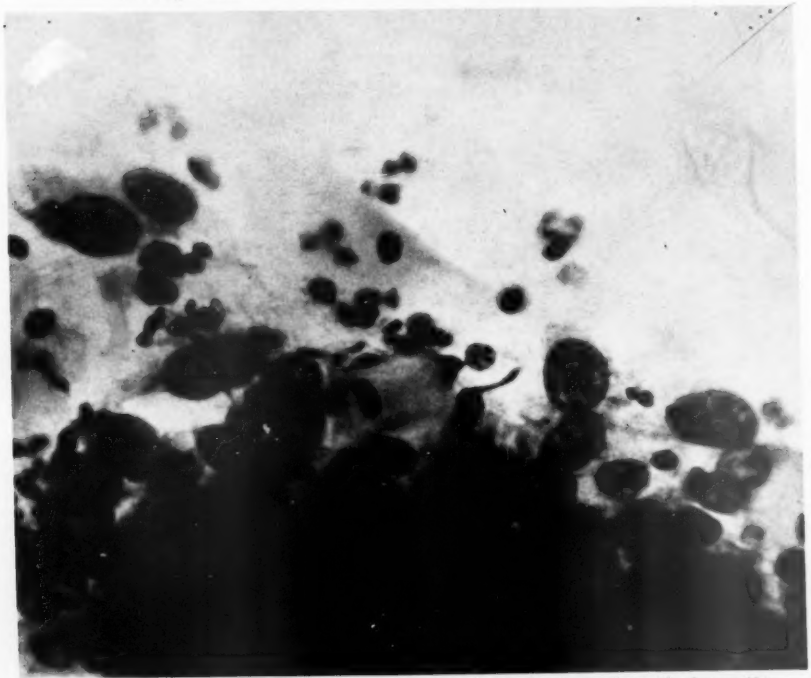


Fig. 3. Dense complex of mostly cytoplasm—free hyperplastic nuclei with chromatin-lumping.
Histology: regenerative epithelium with marked proliferation.

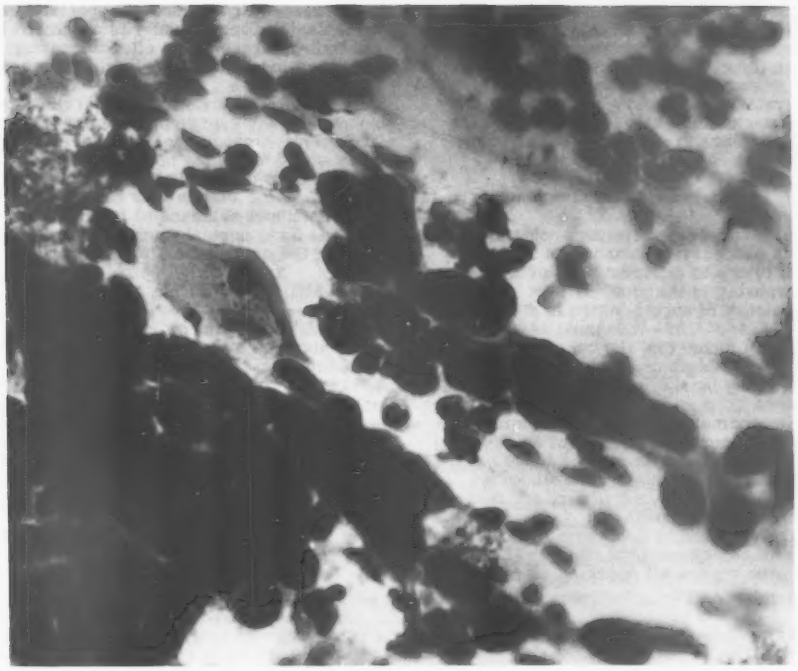


Fig. 4. Markedly hyperchromatic "tumor-cells."
Histology: marked inflammation with inflammatory infiltrated metaplastic changes. No malignancy.

Diagnostic difficulties appear in the differentiation only if the cells of proliferative epithelium or inflammatory or irritative metaplasia are in evidence in the smear. It then comes to superficial dyskaryosis or, more frequently, to basal dyskaryosis (which may alone indicate the presence of a malignancy) (4). Due to their approximately even cylindrical shape and stainability with mucus stains (PAS, mucicarmin), the cells often are still recognized to be of metaplastic origin. Complexes with large nuclei, rich in chromatin in a markedly irritated epithelium and advanced degeneration of such cells are hardly distinguishable from those of a malignant epithelium. If a marked polymorphism is present, the definite cytological differential diagnosis may be impossible, at least with a single cytological examination. As already mentioned, such patterns are, however, compared to the frequency of endocervicitis, histologically and cytologically rare.

Bibliography


1. Wied, G. L.: Das Deutsche Gesundheitswesen, 5: 1444, 1950.
2. Wied, G. L.: Am. J. Clin. Path. 28:232, 1957.
3. Wied, G. L.: Acta Cytol. 2:49, 1958.
4. Zinser, H. K.: Proceedings, Meeting of the Austrian Cancer Society. May, 1959.



GUILLERMO TERZANO
Buenos Aires, Argentina


Exfoliative cytology can be considered as a procedure that provides good information about inflammatory processes. Under the influence of inflammation the columnar epithelial cells may undergo changes similar to those described for cervicitis and endocervicitis: anisocytosis, anisokaryosis, bizarre cells, abnormal vacuolization, dyskaryosis and/or evidence of squamous metaplasia.

The number of erythrocytes, leukocytes, pyocytes, histiocytes and the morphological aspect of the cells lead to the correct diagnosis.

## DISCUSSION

CLARICE DO AMARAL FERREIRA, Rio de Janeiro, Brazil:

I would only like to emphasize some characteristics of those cells exposed to inflammatory processes such as the chromatin pattern in this condition. The clusters of endocervical cells in endocervicitis are usually greater than we observe in normal cases and the nuclei are hyperchromatic. The chromatin sometimes maintains its special distribution on the nuclear membrane,

but to a greater degree, sometimes with a uniform granular distribution of the chromatin, which is not commonly seen in endocervical cells. In addition, there is an increase of leukocytes (many of them degenerated), histiocytes and a type of mucus that has in the estrogenic phase its typical crystallization disturbed because of the failure of its physical-chemical balance. These are the common cytological findings in non-specific endocervicitis.

WARREN R. LANG, Philadelphia, Pennsylvania, U. S. A.:

The ever present, overwhelming problems as I see them are what is endocervicitis and does not the term mean different things to different people? Von Haam's definition probably is the most correct one semantically. The frequency of such a lesion is difficult to determine, but in its "pure" form it is most likely of significance in infertility. I agree that the cause is most frequently coccal but probably not gonorrheal in origin. Some day perhaps we may be routinely culturing various viruses from this area.

The cells desquamated from the anatomical endocervix are usually columnar in nature, but as demonstrated by Ober (1), squamous cells may be found especially in older women. With inflammation well-known changes in the cells of the endocervix occur, and these have been detailed by the main speakers.

What is surely needed is a uniform and comprehensive terminology of benign cervical changes. Only in this way can we be sure we are all speaking on common ground.

Bibliography

1. Ober, K. G.: German Med. Monthly 4:77, 1959.

LUIS MONTALVO RUIZ, Madrid, Spain:

Our findings in cervicitis agree with Gaudefroy's findings. In some cases the only finding in cervicitis is a large amount of very well-preserved polynuclear leukocytes with a moderate amount of histiocytes.

ERICA WACHTEL, London, England, U. K.:

I agree with the three main speakers that endocervicitis is a common condition readily diagnosed in vaginal smears. Since the lesion may not extend beyond the squamo-columnar junction and thus be invisible on inspection, a definite cytological diagnosis can be a great help to a gynecologist.

The typical smears exhibit an inflammatory pattern characterized by the exfoliation of varying numbers of endocervical cells showing signs of degeneration and inflammation, especially in their nuclei. The squamous epithelium may or may not appear normal depending on the presence or absence of co-existing ectocervicitis and metaplasia. There usually is a marked leukocytosis, with the individual pus cells often appearing in a poor state of preservation, and mucus is often present. Von Haam's statement that a Trichomonas vaginalis infestation is rarely found in cases of endocervicitis does not correspond with my experience. Smears from patients with acute Trichomonas vaginalis vaginitis have almost invariably shown evidence of co-existing cervicitis. This applied only to patients where Trichomonas vaginitis was present. Some women may harbor trichomonads as saprophytes without evidence of inflammatory changes. Such patients have normal smear patterns except for the presence of the organisms.

ALEXANDER MEISELS, México, D. F., México:

The not uncommon endocervical hyperplasia presents such a typical cytological appearance that little confusion can arise from it. The sheet-like desquamation of large, often pale, nuclei surrounded by shreds of scarce cytoplasm is easily recognized. Sometimes, however, variations in size of the nuclei and increased chromatic activity can be exaggerated enough to cause serious concern as to the possible malignant nature of the cells. Absence of vacuolization in the cytoplasm and great similarity of the cells inside the group usually permit a correct diagnosis. Repeated smears may be necessary, however, for a satisfactory evaluation.

## CLOSING REMARKS

EMMERICH VON HAAM:

Dr. Ferreira's observation of the effect of the inflammatory processes upon the chromatin pattern of the endocervical cells is correct, but it is much more difficult to recognize than the usual effect of inflammatory conditions upon epithelial cells. I agree with Meisels that the differentiation between degenerated endocervical cells and histiocytes is quite difficult at times.

GUILLERMO TERZANO:

There is little that we can add to close this discussion. As a matter of fact we do not have in our series actual cases in which evidence of inflammation can be considered as being strictly within the limits of the endocervix, but cases in which both, the exocervix and the endocervix, are involved to a certain extent.

The picture of cytology in endocervicitis (extensive desquamation of normal and abnormally featured cells and the background of inflammation) was made upon the study of the smears of the specimens obtained by aspiration into the cervical canal.

# CYTOLOGY OF ENDOCERVICAL HYPERPLASIA AND RESERVE CELL HYPERPLASIA

ARTURO ANGEL ARRIGHI
Buenos Aires, Argentina

ENDOCERVICAL HYPERPLASIA:

We can observe this pathological picture in various circumstances: a) in the normal pregnant women, generally associated with other cervical changes; b) as a congenital condition with hyperplasia of all the elements of the endocervical mucosa with formation of intraperietal cysts situated at different distances from the surface (endocervicosis); c) as a result of mechanical, inflammatory or hormonal (high estrogenic stimulation) causes.

The cervical (scraping or aspiration) smears obtained from these patients are characterized by clusters or aggregates of endocervical cells. Some of them are absolutely normal cells, but the majority of the elements show changes in their nuclei which consist of marked variation in size and shape with a not infrequent alteration of the normal nuclear-cytoplasmic ratio. Of decisive importance to the diagnosis is the preservation of the chromatin pattern of the cells, with a nucleolus and diffuse and dusty distribution of the chromatin inside the clear nucleus.

Such changes are, otherwise, not pathognomonic of endocervical hyperplasia, and we are notable to perform the diagnosis by means of exfoliative cytology.

RESERVE CELL HYPERPLASIA:

We have never observed in the smears cells that we can clearly differentiate and classify as reserve cells. The retrospective examination of the smears of cases with the diagnosis of reserve cell hyperplasia, done histologically, did not permit the recognition of a significant cytological pattern.

JEAN A. de BRUX AND J. DUPRÉ-FROMENT
Paris, France

Histologically, the hyperplasia of the reserve cells and the hyperplasia of the endocervical mucosa cannot be compared: The one is simply the exaggeration of normal cellular elements, whereas the other is the result of the replacement of the columnar epithelium by another epithelium of metaplastic origin but whose elements remain undifferentiated, piled up without stratification, and immature, hence often presenting an aspect alarming for the untrained eye.

Cytologically, however, the elements exfoliated from these two lesional types resemble each other, and sometimes pose a difficult differential problem, for in both cases hyperplastic cells are involved:

A. The cell of the endocervical hyperplasia is columnar and hypertrophic, but very often in process of transformation by metamorphosis or by metaplasia.
B. The hyperplastic reserve cell is of indefinite morphology, because it is undifferentiated, being neither columnar nor epidermoid.

Hence there is a difficulty in diagnosis.

A. The endocervical hyperplasia is rather a clinical concept and a histological fact than a cytological entity. It is expressed on the pathological slides by an exaggeration and excessive ramification of the endocervical fringes, whose stroma is edematous and inflamed, and whose border epithelium is multistratified, the cells having a nucleus whose chromatin is increased but regularly distributed, and a cyanophilic cytoplasm. The glands are often hypersecreting.

Hyperestrogenism, inflammation and obstetrical factors are the causes usually invoked to explain hyperplasia of the endocervical mucosa; and these causes, moreover, often coexist.

On the smear, the aspect is not very characteristic and is just as likely to elicit a diagnosis of untransformed endocervical polyp; all depends on the way in which the sampling is done:

If taken with only a light stroke, the slide shows a moderate quantity of endocervical cells and of naked nuclei.
If pressure is exerted, this will remove shreds of endocervical mucosa more hyperplastic and more florid than that seen in the endocervical polyp.

The endocervical hyperplasia usually shows clumps of cells rather loosely agglomerated (as if ready to dissociate), short fringes of mucosa, isolated cells, and especially a large number of naked nuclei dispersed in disorder or in trails.

These columnar cells show various aspects:

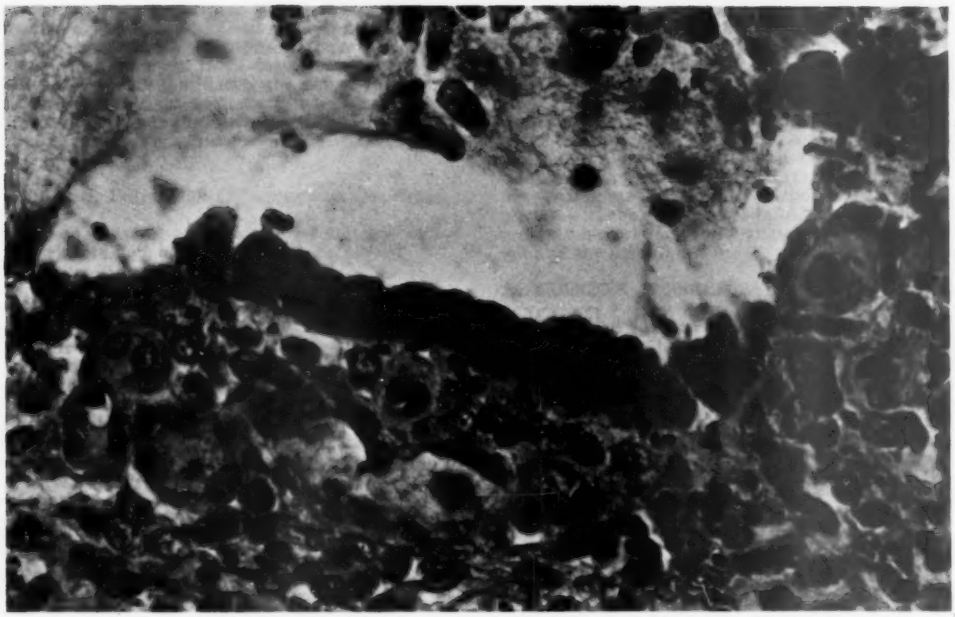


Fig. 1. Cylindrical endocervical cells in the process of metamorphosis.

Certain of them have a very hyperplastic nucleus with chromatin finely distributed but clearly outlined; the nuclear membrane is accentuated, and the nucleoli are prominent. The cytoplasm is cyanophilic, somewhat enlarged, and generally rounded; sometimes one or more small secretory vacuoles are noted (Fig. 2).

In certain other cells, the nuclei are swollen, and the chromatin, very finely distributed, appears as if distended by the edema (Fig. 3).

In still other cells, the chromatin structure is effaced, showing no reticular design, grayish or hyalin (Figs. 4, 5).

Nowhere on these smears can there be found elements of epidermoid metaplasia, as are seen in the case of polyps and ectropions. The very frequent inflammation, on the other hand, causes a lysis of the cytoplasm and the appearance of naked, shrunken nuclei.

B. Hyperplasia of the reserve cells, or "active, undifferentiated and immature metaplasia."

The latter term describes exactly the form the lesion takes on the pathologist's slide. It is in fact a metaplasia of a very special type, very alarming for anyone with little experience:

excessive multiplication and density of the nuclei, whose chromatin structure is grainy and thick;

disorderly packing of the cells, which pile up without any tendency towards stratification until the very upper layers, and without organoid structure;

total absence of cellular maturation;

presence, rather high in the epithelium, of mitotic figures (Fig. 6).

Such are the characteristics which, on the histological slide, may pass as a carcinoma in situ.

The first term, "hyperplasia of the reserve cells," defines with exactitude the indifferent character of the elements, and their absolutely benign nature. And it is on the smear that the pathologist's error is brought to light. Two types of exfoliated elements are found:

1. Certain of them have an aspect of roughly metaplastic cells;
2. Others are more or less dyskaryotic, and correspond to a suggestion of mechanical maturation of the surface elements.

1. The cells of this first type exfoliate in small shreds in which the cells are indistinctly outlined and sometimes almost syncytial, with cytoplasm tattered, stretched and very cyanophilic. These facts explain the difficulty in classifying the cell: hyperplastic endocervical cell in metamorphosis or metaplastic parabasal cell (Fig. 7).

The nucleus is central, voluminous, decidedly hyperplastic, and sometimes swollen. The chromatin is regular, but its grains are accentuated, sometimes larger and denser than normal. The nucleoli are generally prominent. Sometimes the nucleus is still more bloated, the chromatin appearing vacuolized and the grains being sparsely but well distributed (Fig. 8).

In addition to these images of cells in active hyperplasia, there are also seen a certain anisonucleosis and a slight inversion of the normal nuclear-cytoplasmic ratio (Fig. 9).

2. The second type of cell exfoliated appears to undergo a beginning of maturation, but in reality what takes place is only a phenomenon of suffering: The cytoplasm may be elongated and, sometimes, faintly eosinophilic. The nucleus is dyskaryotic: incompletely retracted, with incisions and more or less irregular con-

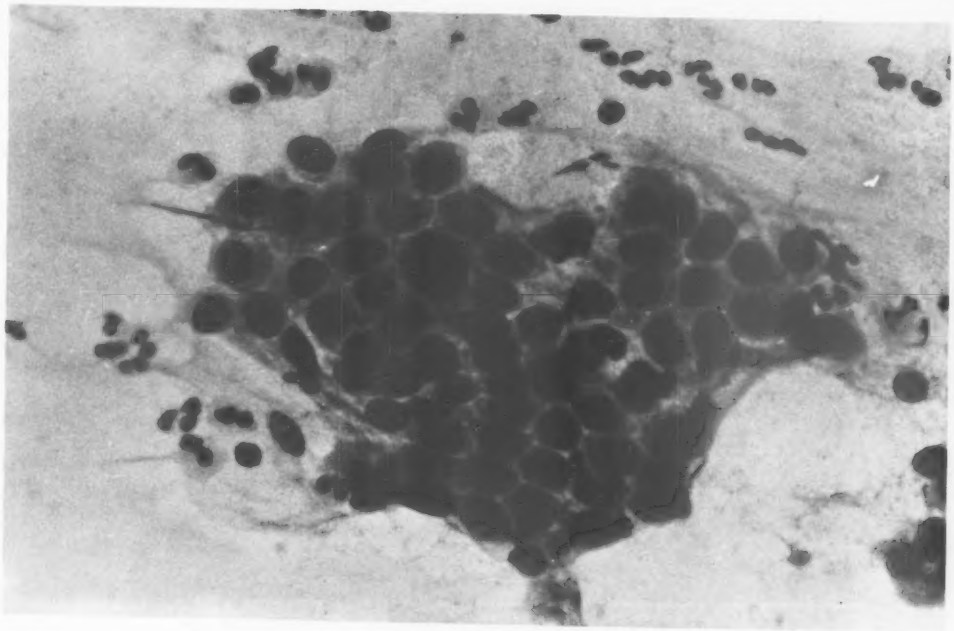


Fig. 2. Hyperplastic endocervical cells displaying anisonucleosis.

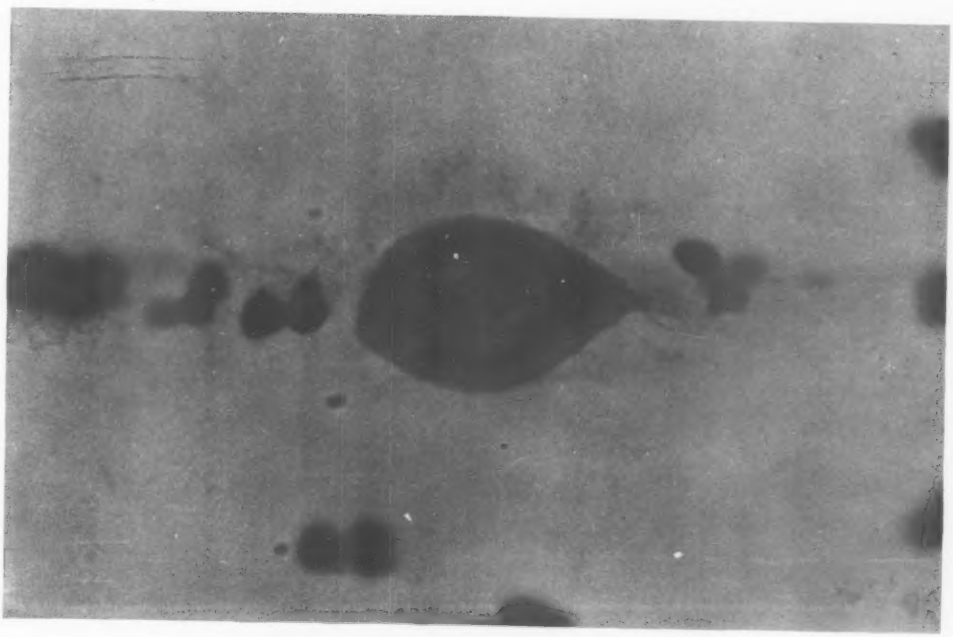


Fig. 3. Nucleus from a simple hyperplasia (oil immersion).

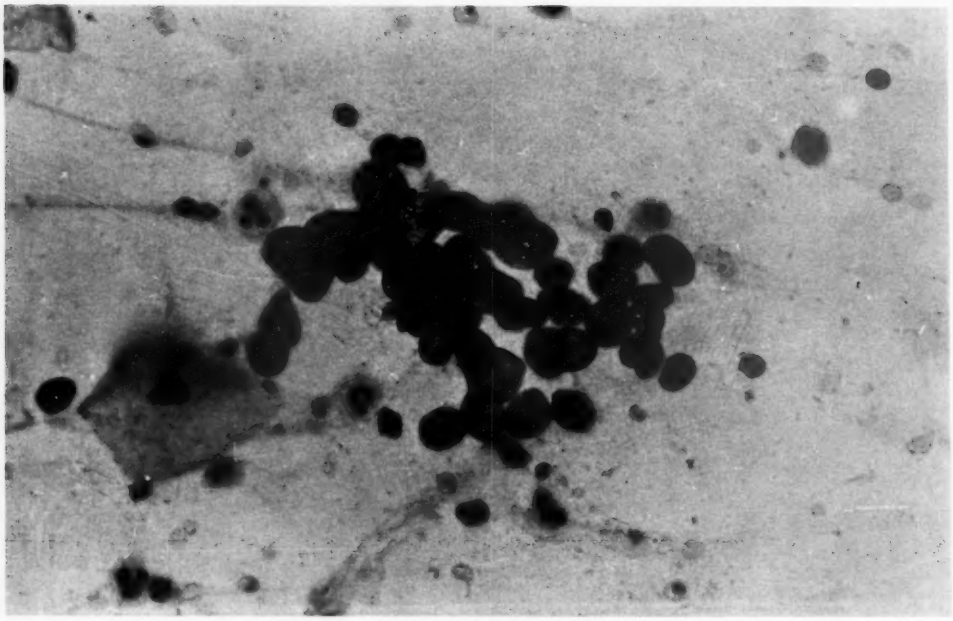


Fig. 4. Naked hyperplastic endocervical nuclei.

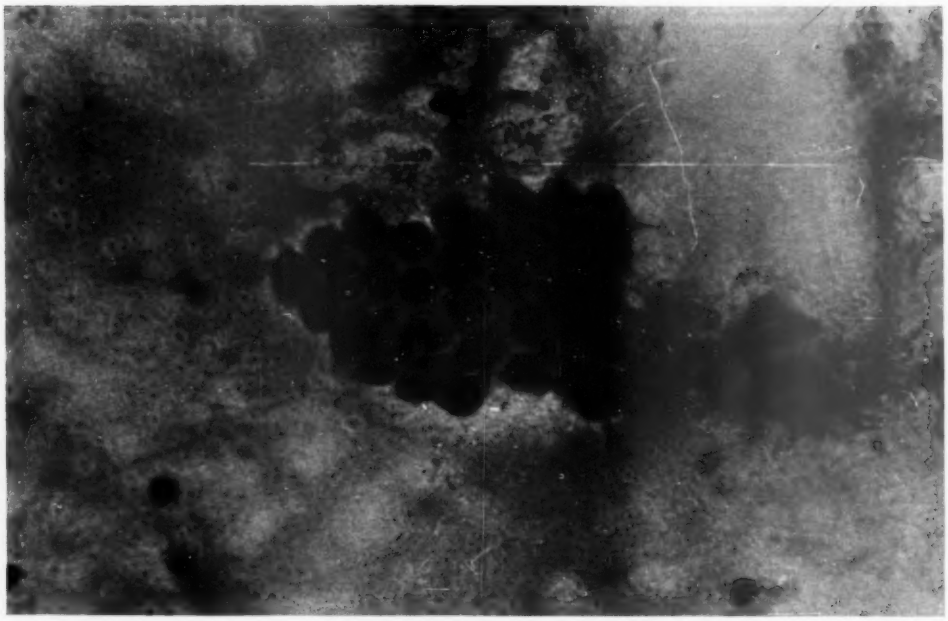


Fig. 5. Cluster of hyperplastic endocervical cells.

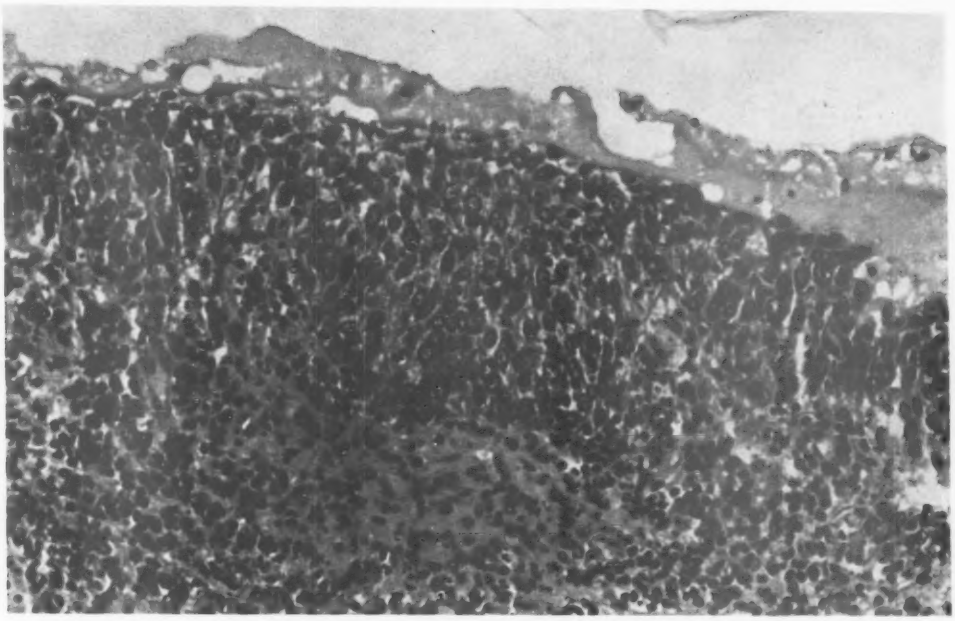


Fig. 6. Reserve cell hyperplasia.

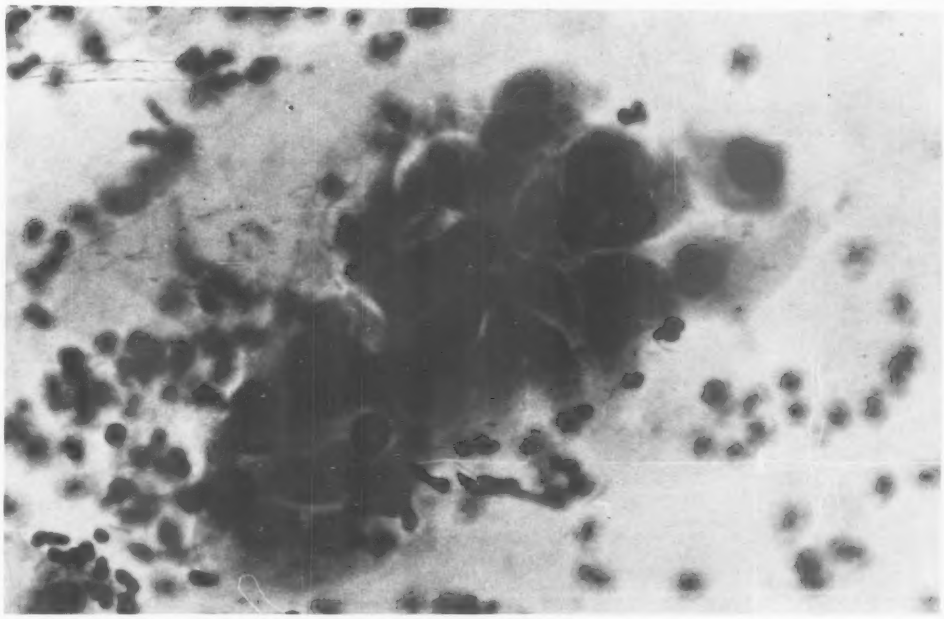


Fig. 7. Reserve cell hyperplasia: scant chromatin.

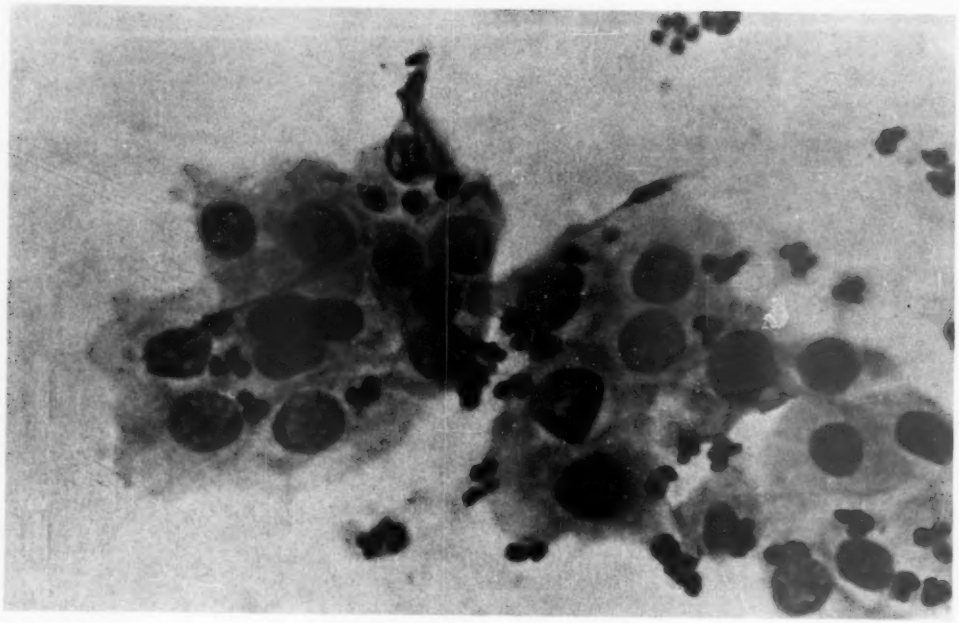


Fig. 8. Reserve cell hyperplasia.

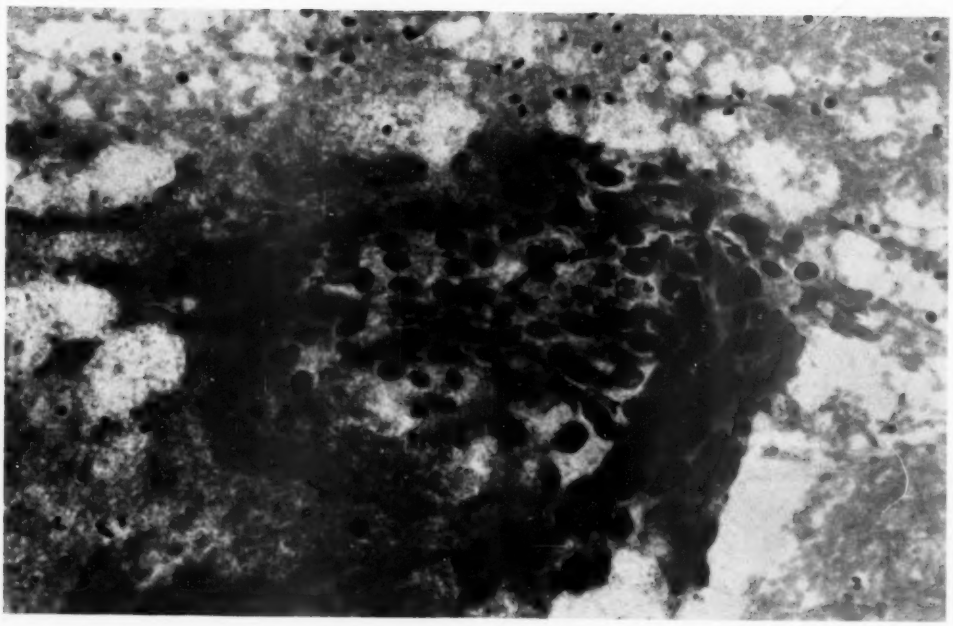


Fig. 9. Reserve cell hyperplasia.

Fig. 10. Reserve cell hyperplasia. A cell cluster of dyskaryotic type in the process of disquamation.

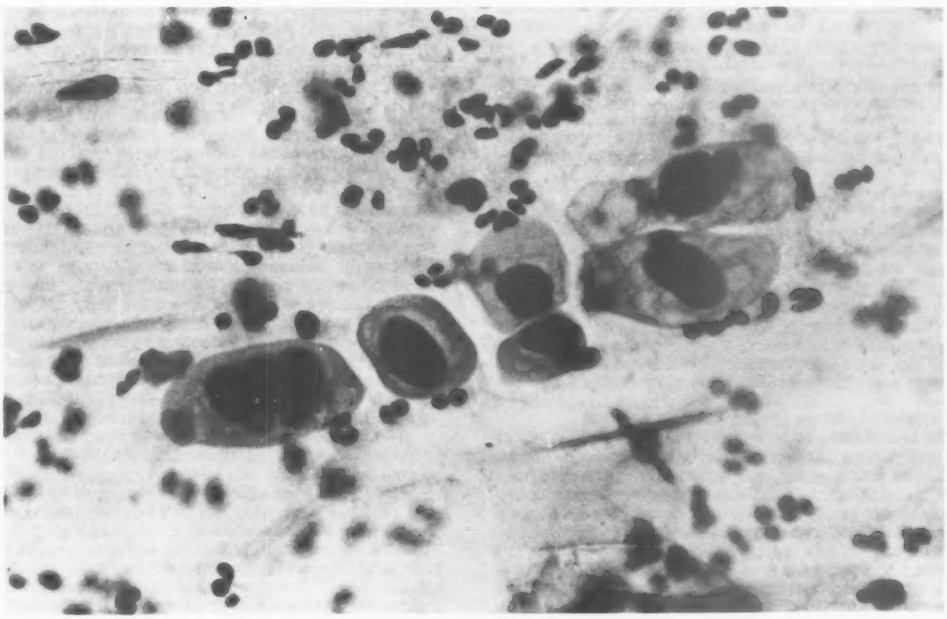


Fig. 11. Reserve cell hyperplasia: Dense but regular pattern.

tours. The chromatin nevertheless remains regular. In certain cases, however, it may become opaque, giving to the nucleus a dysplastic aspect (Figs. 10, 11).

Although the diagnosis of the benign nature of the change is easily deduced, that of the lesional variety, on the contrary, is sometimes difficult, in particular, the differentiation from the ectropion and the endocervical hyperplasia.

The small shreds having the aspect of endocervical cells in metamorphosis (but cyanophilic and not polychromatophilic), the absence of naked nuclei, and the lack of dispersion of these elements (which are agglomerated in patches), call for a diagnosis of hyperplasia of the reserve cells.

However, the possible presence of dyskaryotic and dysplastic cells is sometimes confusing and raises the question of a lesion of greater severity. In this case the chromatin should be minutely studied: its regularity remains the final test of the benign cellular entity.

The active undifferentiated immature metaplasia is often confusing for the pathologist, who must recognize and systematically analyze the cellular nuclei before mentioning carcinoma in situ. He can therefore give a diagnosis only after a careful study of the smears.

From every point of view, this entity is of extreme interest in the question of the histogenesis of the cervical lesions.

## DISCUSSION

MARIA S. BLANCO DE DEL CAMPO, Palo Alto, California, U. S. A.:

I would feel that "reserve cell hyperplasia" and "endocervical hyperplasia" are not desirable terms for cytological use. I prefer the term "dysplasia" with qualifying adjectives to cover both these above terms. I accept Arrighi's viewpoints.

ALEXANDER MEISELS, México, D. F., México:

The not uncommon endocervical hyperplasia presents such a typical cytologic appearance that little confusion can arise from it. The sheet-like desquamation of large, often pale, nuclei surrounded by shreds of scarce protoplasm is easily recognized. Sometimes, however, variations in size of the nuclei and increased chromatic activity can be exaggerated enough to cause serious concern as to the possible malignant nature of the cells. Absence of vacuolization in the cytoplasm and great similarity of the cells inside of the group, usually permit a correct diagnosis. Repeated smears may be necessary, however, for a satisfactory evaluation.

## CLOSING REMARKS

ARTURO ANGEL ARRIGHI:

The matter involves two problems. The one of nomenclature; the possibility of relating cytological pictures to pathological ones. The other concerning the differentiation of these images (reserve cell hyperplasia, basal cell hyperplasia and dysplasia) from malignant images.

We believe that both problems have had adequate answers in the discussion.

JEAN A. BRUX:

I am altogether in agreement with Meisels, but I cannot accept the term "dysplasia" for the two dystrophic lesions: hyperplasia of reserve cells and endocervical hyperplasia.

The endocervical hyperplasia is simply an exaggeration of a normal process, arising from phenomena of poorly resorbed congestion and edema, intensified by more or less marked inflammation in the endocervical mucosa.

The hyperplasia of the reserve cells is a process of metaplasia: the reserve cells, instead of becoming differentiated into columnar cells or epidermoid cells, remain undifferentiated and pile up, thus forming a curious epithelium, extremely interesting for an understanding of the histogenesis of the irregular dysplasias and of the so-called carcinoma in situ. We have designated as "active, undifferentiated and immature metaplasia" the process resulting in this peculiar epithelium.

# CYTOLOGY OF ENDOCERVICAL POLYPS

JEAN A. de BRUX AND J. DUPRÉ-FROMENT
Paris, France

Histologically, two sorts of polyp are to be distinguished: the adenomatous polyp and the fibrous polyp of long standing.

The polyp is a pediculated formation, with a dense and fibrillary or fibrous stroma and contains mucus-producing glands, sometimes hypersecreting and adenomatous. Peripherally, it appears to be formed from numerous endocervical fringes bordered by a hyperplastic and sometimes secreting columnar epithelium.

A. The cytological picture of the adenomatous polyp presents no problem, for its exfoliation is abundant and corresponds perfectly to the histological aspect. There are found, in fact, large shreds of mucosa—true fragments of tissue which may sometimes occupy two or three microscopic fields.

The cytoplasmic limits are not visible, and there is noted a monomorphous patch of nuclei more or less crowded, rounded and very regular. The chromatin is dense but finely specked; a nucleolus may sometimes be seen, and the nuclear membrane is accentuated. Rather rarely there may be a slight anisonucleosis, usually in the patches of lesser extent.

Between the shreds there are found numerous masses, coherent or incoherent, and in process of dispersion, of varying size but all of identical morphology. There are also seen streaks of naked nuclei, often swollen, some of which seem emptied of their contents. Such nuclei then show what seems to be an amorphous, opaque greyish substance, or may be reduced to a few chromatin grains separated by large vacuoles (Fig. 1).

When the columnar border is secretory, or when a mucous gland communicates with the exterior as a result of abrasion, there appear on the smear endocervical cells with vacuolized cytoplasm whose nucleus is pushed toward the periphery. In certain cases, which usually co-exist with an inflammation of the subjacent stroma or with the appearance of the polyp at the external orifice of the cervix, there occurs an eosinophilic or metaplastic transformation of the columnar border.

On the smear, these phenomena are expressed by the appearance of transformed endocervical cells of "racquet" form, with polychromatophilic cytoplasm, and whose prominent and violet-tinged nucleus has decidedly assumed hyperplastic characteristics contrasting with the less florid aspect of the other endocervical nuclei. These parabasal cells of metaplasia resemble them strongly but are larger and sometimes show a frankly cyanophilic cytoplasm (Fig. 2).

Three sorts of criteria permit the cytological diagnosis of adenomatous polyp, whether it be transformed or not:

1. a quantitative notion related to the abundance of the columnar elements encountered, in particular the finding of true biopsic specimens of mucosa, with a certain dispersion of the cellular elements across the entire smear;
2. the localization of these formations on the endocervix, unless the polyp protrudes at the external cervical orifice;
3. a qualitative notion, represented by the hyperplastic character of the nuclei.

This is somewhat special, however, since the chromatin in this case seems less florid than with the ectropion or the endocervical hyperplasia. In this last lesion, the cells are in loosely agglomerated clumps, ready to dissociate, with a large number of naked nuclei in trails or dispersed in disorder. Certain of these cells have a hyperplastic nucleus and an enlarged cyanophilic cytoplasm. Others have a swollen, distended nucleus. As contrasted with the ectropion, no epidermoid elements are seen (Fig. 3).

One grave error is possible, but it may always be avoided if care is taken to examine the smear attentively and in its entirety. Certain very differentiated endocervical smears may exfoliate in the form of large patches of columnar cells with hyperplastic and very regular nuclei, whose chromatin is only slightly active, with prominent nucleoli. It is the finding of an anisonucleosis, always easily located, and especially, of franker anomalies, which permits the diagnosis of cancer; in particular, the existence of small papillary clumps of columnar cells whose cytoplasm is often vacuolized.

B. The fibrous polyp, whose sclerous stroma is almost or completely devoid of glands and whose border is composed of a quasi-atrophic epithelium, exfoliates relatively few cells. One notes a few shreds of mucosa, whose crowded nuclei are of small size, very regular, with shrunken chromatin of dusty appearance. There is no longer a nucleolus, and the nuclear membrane is barely visible. The naked nuclei are retracted, often pyknotic. Certain patches are composed of contiguous cells with accentuated cytoplasmic limits; the central nucleus is retracted, dense, sometimes irregular and chromatic, and surrounded by a pale halo. This aspect may be interpreted as an atrophic degeneration.

This cytological picture is moreover difficult to

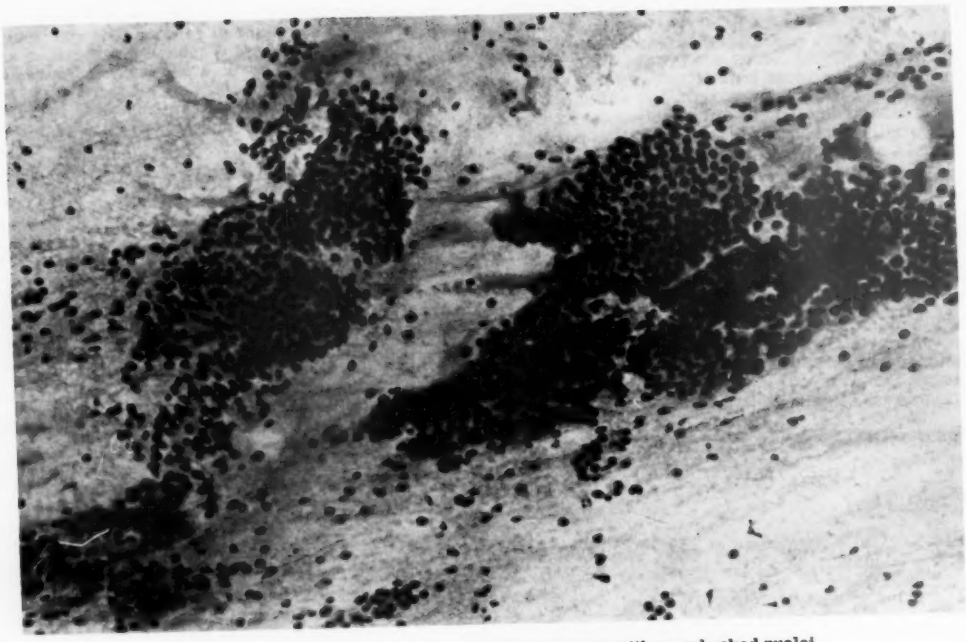


Fig. 1. Endocervical polyp, large clumps of mucosa with round naked nuclei.

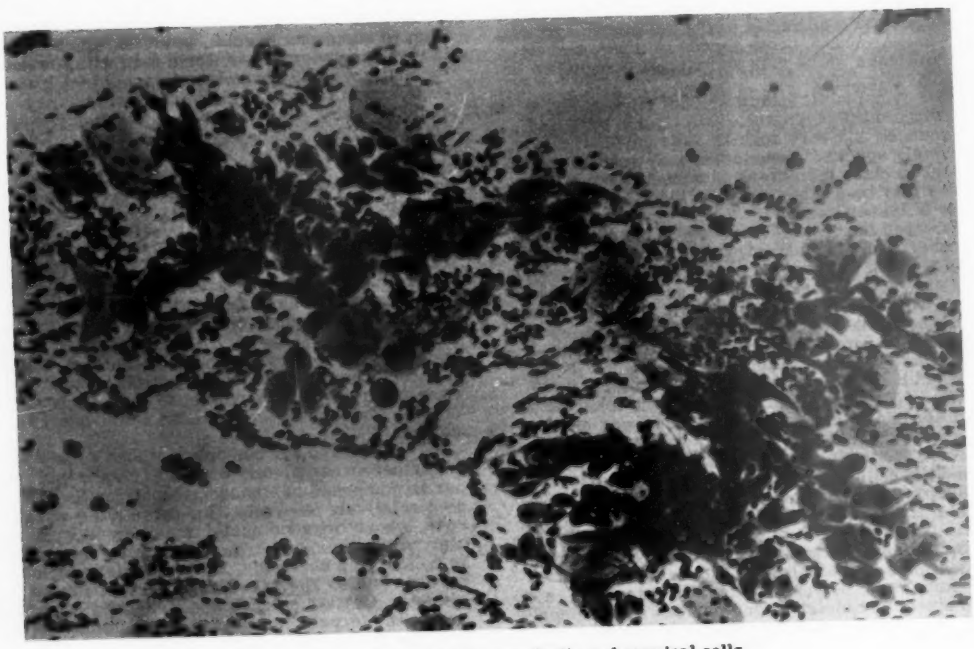


Fig. 2. Metamorphosed and metaplastic endocervical cells.

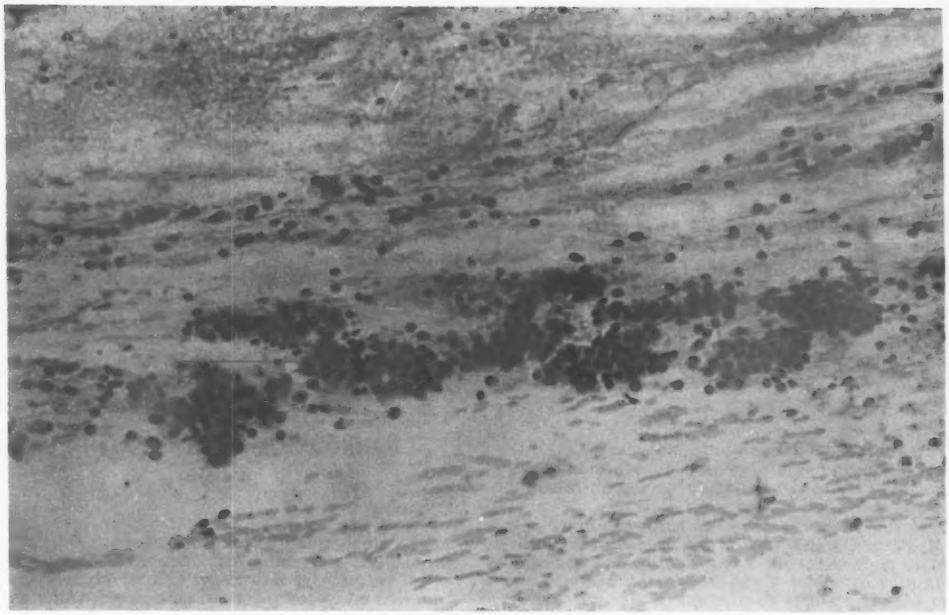


Fig. 3. Endocervical hyperplasia, dispersion of cells beside clumps.

interpret and to distinguish from that of an inflammatory endocervix, in which the columnar elements, more abundant than normally, show numerous signs of suffering. The difficulty is increased by the fact that often the polyp is the seat of inflammation and even of a partial necrosis of its surface. Hence, generally speaking, the fibrous polyp appears cytologically as an ill-defined lesion, and one is often in a difficulty to reach a conclusion, not having sufficient cellular arguments at one's disposition to make a diagnosis with certainty.

## DISCUSSION

WARREN R. LANG, Philadelphia, Pennsylvania, U. S. A.:

Drs. de Brux and Dupré-Froment distinguish between two types of polyp; the adenomatous and fibrous. The former type is, by far, more prone to exfoliate cells. Since the adenomatous polyp arises from the anatomic endocervix it is covered mainly by columnar epithelium and the corresponding cells are to be found in the cytologic smear. These are not characteristic, however, and a cervical polyp cannot be suspected by the smear technique alone. In common with cervical columnar epithelium in general, epidermization may occur and often does; in such cases one finds atypical cells which are most frequently Class II. Inflammatory changes in a polyp can also modify the cells seen. Malignancy in a cervical polyp is quite rare, but when it does occur malignant appearing cells may also be detected.

MARCEL GAUDEFROY, Lille, Nord, France:

I think the description of de Brux will become generally accepted. In case of a mucous or adenomatous endocervical polyp, the cytological diagnosis is easy if clumps or streaks of cells (biopsy-like specimens) are present.

The diagnosis is more difficult when squamous metaplasia or chronic infection is encountered inducing cellular atypias. In these cases the cytologist is often uncertain. In my opinion, if there is doubt about the presence of cancer because the cellular criteria are very poor and inconclusive, one will be almost always correct in answering: no malignancy, probably transformed endocervical polyp.

LEOPOLD G. KOSS, New York, New York, U. S. A.:

I am afraid that I cannot agree with de Brux and Dupré-Froment that "the cytological picture of the adenomatous polyp presents no problem." Admittedly, it should not be confused with cancer but the cytologic diagnosis of an endometrial polyp is not possible in my experience. However, the purpose of this discussion is to point out that in the presence of a polyp and probably because of pressure of the polyp on adjacent epithelial structures, the cervical smears may contain small, flat, anucleated cells which may be quite misleading to an inexperienced eye. Such cells originate usually from the surface of a keratinized focus of squamous metaplasia of the endocervix. They should not be confused with cancer.

LUIS MONTALVO RUIZ, Madrid, Spain:

Dr. de Brux has really exhausted the subject concerning cytology of polyps and we agree with him, but we do not always see this cytological pattern in the smears, particularly in the fibrous polyps which have scanty exfoliation. On the other hand, according to our experience, of all the benign lesions of the cervix, the polyps are the ones in which cellular atypia are found sometimes to such an extent that one is doubtful of whether or not they are neoplastic cells. We have seen, in

some smears of endocervical polyps, nuclei with very active chromatin, thickening of the nuclear membrane, little or no cytoplasm, sometimes anisonucleosis. In many of these cases the biopsy was negative for malignancy. Occasionally the smears are completely negative, without atypia. This does not mean that there is no polyp, but the specimens do not represent exfoliated cells from the polyp. In such cases, the cytologic examination should be repeated.

GUILLERMO TERZANO, Buenos Aires, Argentina:

We are unable to describe a definite cytologic pattern in the smears of patients with endometrial or endocervical polyps. In such cases the cells can appear normal (due to concomitant processes) or in polypoid cluster formation, or the smear may be one with uncharacteristic features. Nevertheless, an endocervical polyp can be suspected by exfoliative cytology in those cases in which clusters of endocervical columnar cells are present and in which one has no reason to consider other physiological or pathological conditions that would explain this type of exfoliation.

## CLOSING REMARKS

JEAN A. DE BRUX:

I thank Drs. Montalvo and Terzano, who have answered perfectly the criticisms of Drs. Koss and Lang.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

# CYTOLOGY OF ENDOCERVICAL ADENOCARCINOMA

JEAN A. de BRUX AND J. DUPRÉ-FROMENT
Paris, France

Whereas the various histological aspects of the endocervical adenocarcinomas are numerous and permit the description of particular types, the cytological images, on the contrary, are so few that, based on them alone, one can only make the diagnosis of adenocarcinoma, without generally defining the variety.

We shall not discuss here the morphological criteria of carcinoma: inversion of the nuclear-cytoplasmic ratio, thickening of the nuclear membrane, density and irregular distribution of the chromatin and monstrosities. Several characteristics, nevertheless, are specific of endocervical adenocarcinoma.

The adenocarcinoma of the endocervix is expressed on the smears by elements whose nuclear characteristics are more hyperplastic than carcinomatous (Fig. 1). The nuclei are in fact voluminous but regular and swollen, with fine chromatin at times dense, at other times less dense, but always clear (Fig. 2). It forms a delicate web which may be close-meshed or loose and sparse, and here and there it is dotted with grains, sometimes dispersed, sometimes adhering to the nuclear membrane, which, given the chromatin characters, is simply accentuated (Figs. 3, 4). The nucleoli, on the other hand, are very prominent.

The cytoplasm, rather large, tends to become rounded. It is slightly cyanophilic or polychromatophilic, and often contains one large vacuole, or numerous small cavities (Figs. 5, 6).

These cytological findings are hardly suggestive

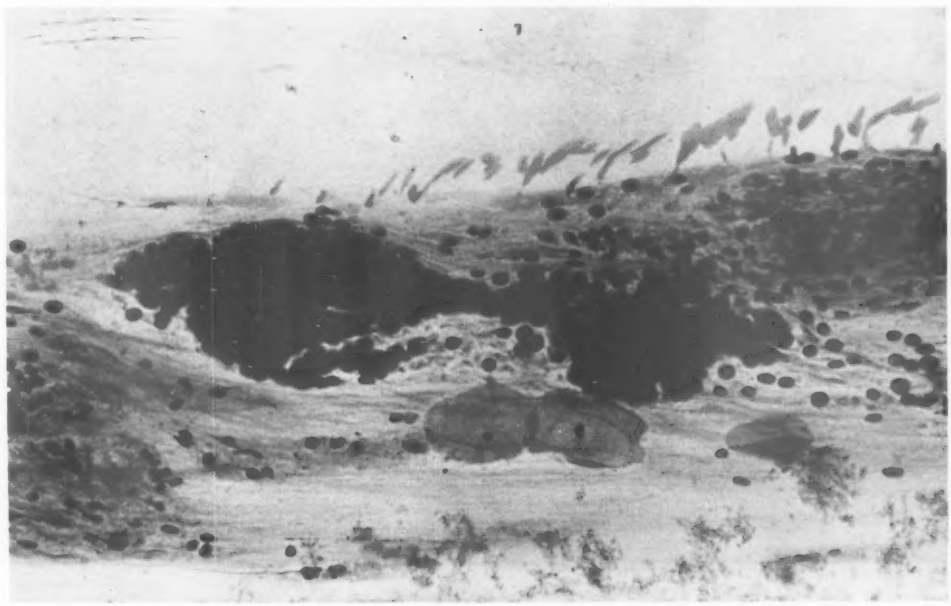


Fig. 1. Adenocarcinoma of endocervix: polypoid clump with naked nuclei with grainy chromatin.

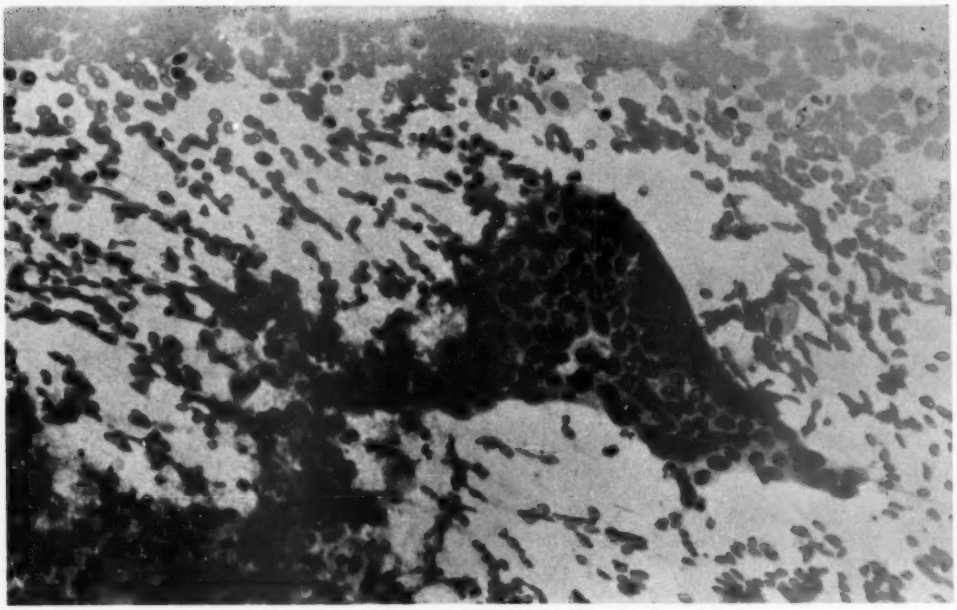


Fig. 2. Clump of the same appearance with mitosis, anisonucleosis and grainy chromatin.

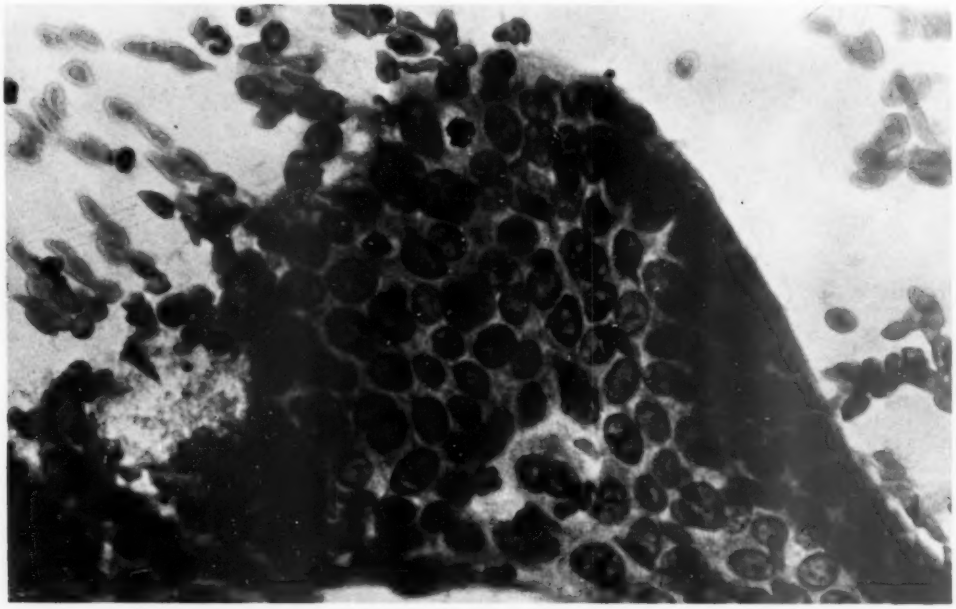


Fig. 3. Enlargement of Fig. 2.

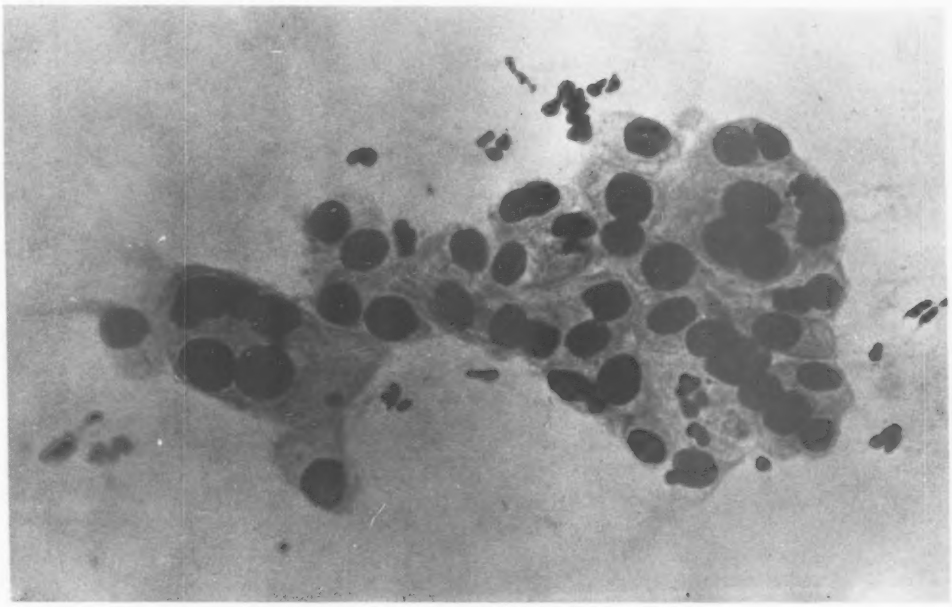


Fig. 4. Polypoid type of adenocarcinoma of the endocervix.

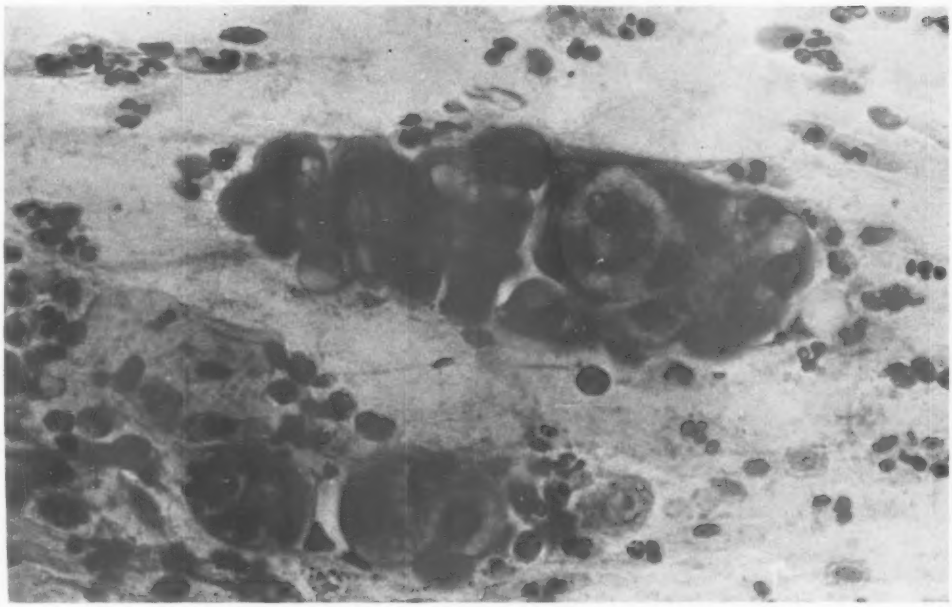


Fig. 5. Papillar clump of cancerous cells.

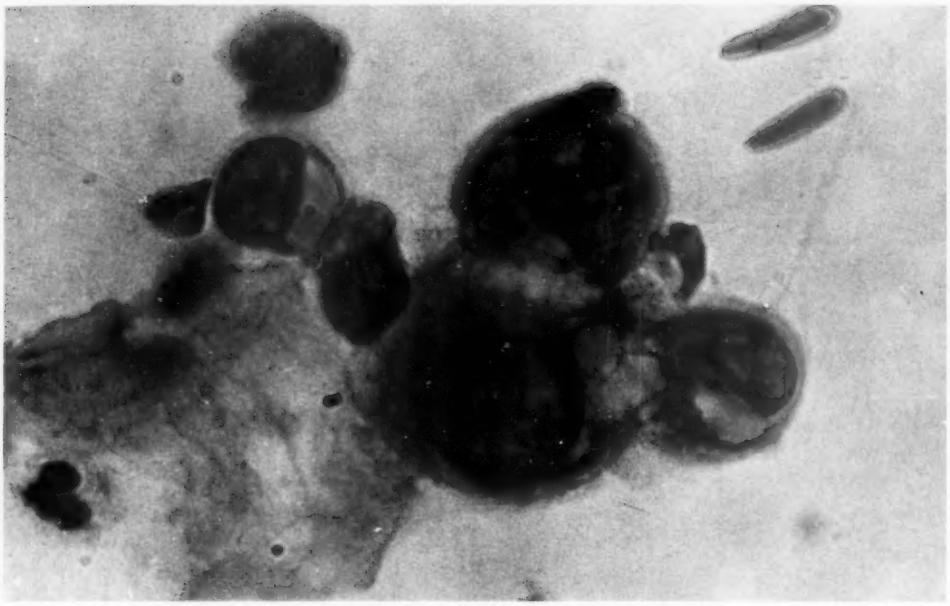


Fig. 6. Oil immersion view of Fig. 5.

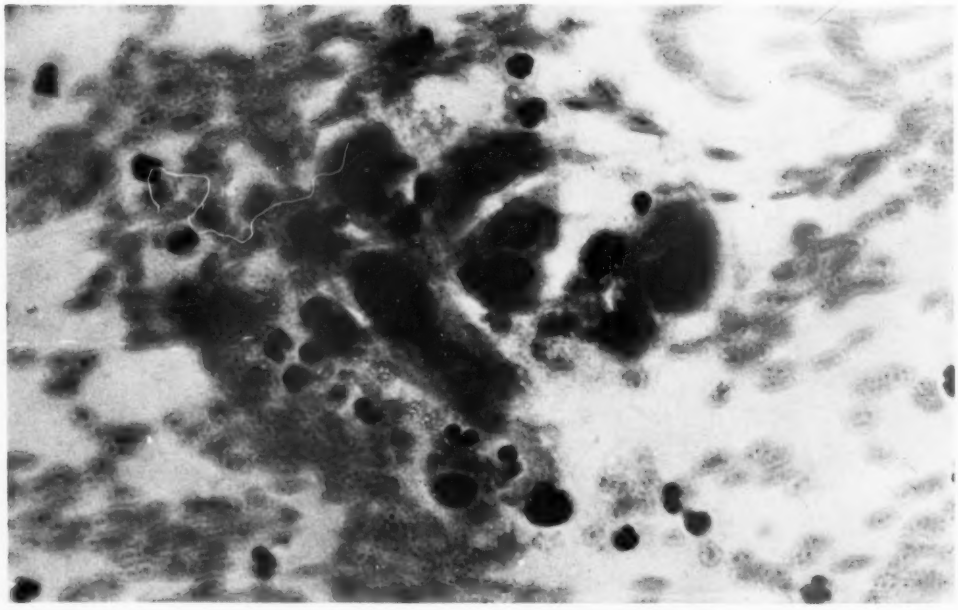


Fig. 7. Clump of adenocarcinoma cells. Note double nuclei with very prominent nucleolei and relatively clear chromatin.

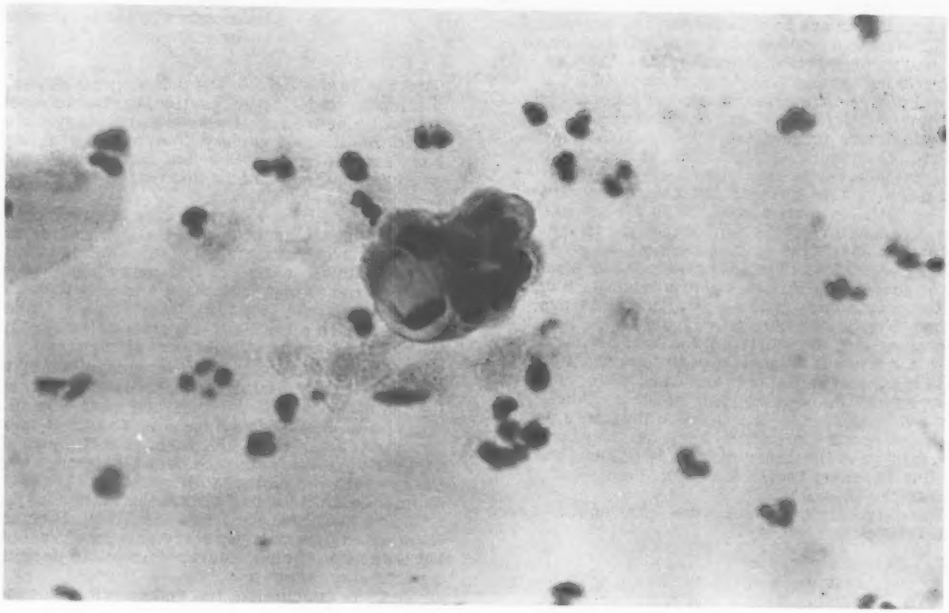


Fig. 8. Retracted nuclei and cytoplasmic vacuolization.

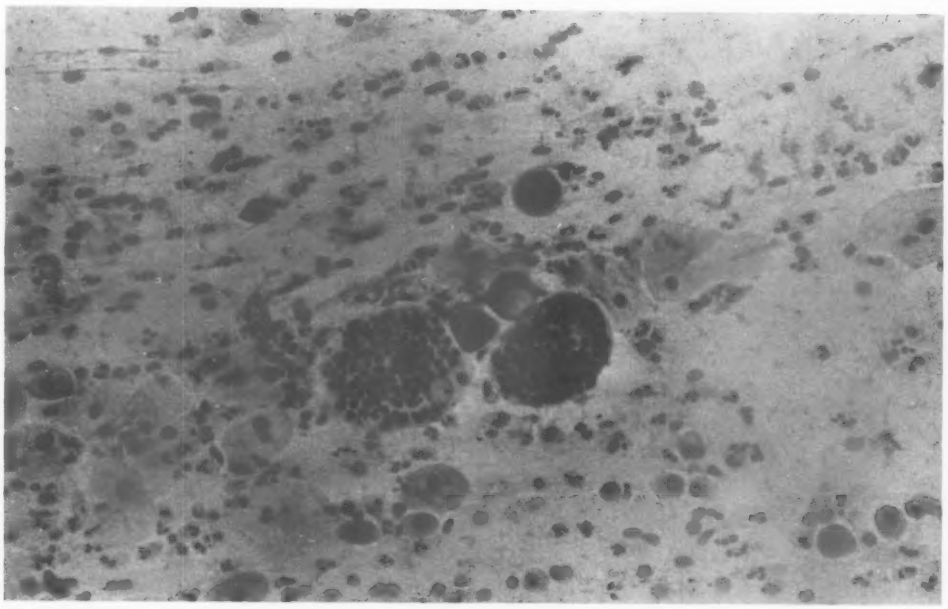


Fig. 9. Cells of parabasal type and macrophagia.

of malignancy and, based on only a single superficial examination would lead one to the diagnosis of polypoid hyperplasia of the endocervix. In order to avoid an error, one must search for certain anomalies: the cellular groupings evoking the papillary aspect so frequent in the endocervical adenocarcinomas; a marked anisonucleosis; the bi- or tri-nucleation; the size of the nucleoli.

Once having found these anomalies, one will rapidly perceive other facts, in particular, the incisions of the nuclei, which nevertheless remain clear, a few mitoses, and a few images of black, angular chromatin.

The cellular context itself is an aid not to be neglected: A characteristic rather particular to the endocervical carcinomas is the macrophagia of the polynuclear cells by the cells of the adenocarcinoma; we may consider, however, that this is a characteristic in common with the carcinomas of the genital tract (corpus uteri, ovary) (Fig. 9). Numerous polynuclear cells are grouped in clumps in the bloated cytoplasm of the cancerous cell, whose nucleus is pushed back toward the periphery and the laminated cellular membrane. A very characteristic fact is that there is no lysis of the polynuclear cells (Fig. 10). Later, it is the nuclei of the cancerous cells which disappear (perhaps by lysis by the polynuclear ferments), and the dense clumps of polynuclear cells in a fine envelope recall somewhat the aspect of certain parasites (Fig. 11).

The diagnosis of endocervical carcinoma, therefore, is based on:

1. Direct signs
   clearness of the nucleus,
   anisonucleosis and multinucleation,
   prominent nucleoli,
   cytoplasmic vacuolization,
   papillary grouping of the elements, and
2. Indirect signs
   macrophagia of the polynuclear cells by malignant cells.

In the light of the pathological slides, we have attempted to define the particular characteristics of certain cells by means of whose morphology we may conclude the tumoral variety. Two cytological aspects only have seemed noteworthy, that of the double epidermoid and columnar carcinoma and that of the very differentiated papillary carcinomas.

The first variety shows 1) carcinomatous and epidermoid elements of the usual character and 2) cells of columnar type, isolated and not contiguous in clumps; they are small in size, with a bulky nucleus whose chromatin is dense and dark. There is only a thin ring of bluish cytoplasm (Fig. 12). This immature and poorly differentiated aspect corresponds to the canalicular aspect of the Gartnerian adenocarcinoma.

The second variety is recognized by the presence of all the cellular types, isolated or grouped, recalling the endocervical polyp, except that here all the evolutive states may be found from one patch to another—hyperplastic form (Fig. 13), form in metamorphosis (Fig. 14), metaplastic form with parabasal cells disposed in bands—but even on these differentiated forms there are anomalies sufficient to affirm the malignancy.

The clear-cell mesonephrotic carcinomas are manifested by small papillary groups of vacuolized cells, but without sufficient characteristics to permit isolating a particular form.

In conclusion, the endocervical adenocarcinomas have essentially a papillary and differentiated morphology. The elements perhaps exfoliated from a proliferation of Gartnerian origin have a particular morphology.

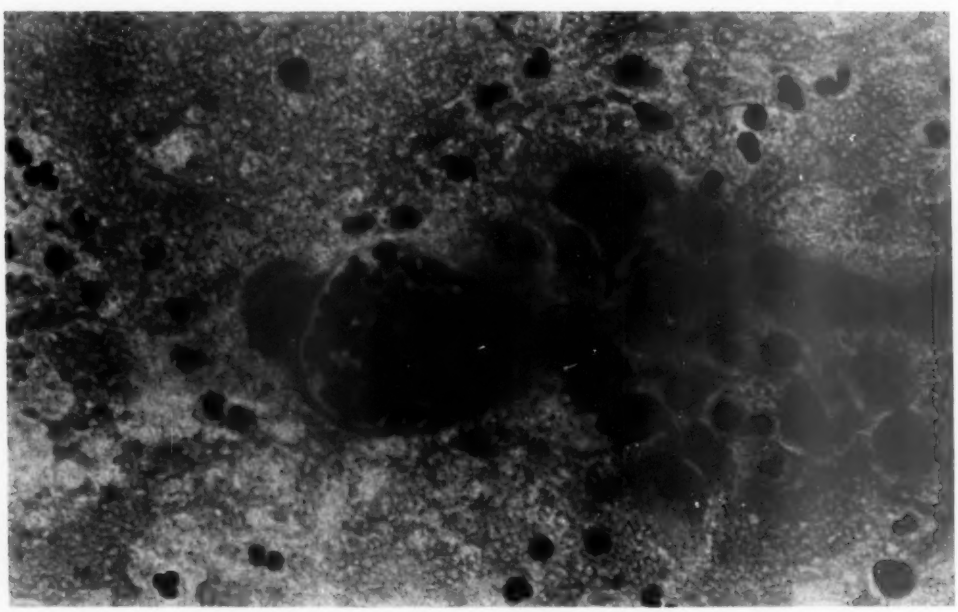


Fig. 10. Endocervical cancer cells, one of which is macrophagic.

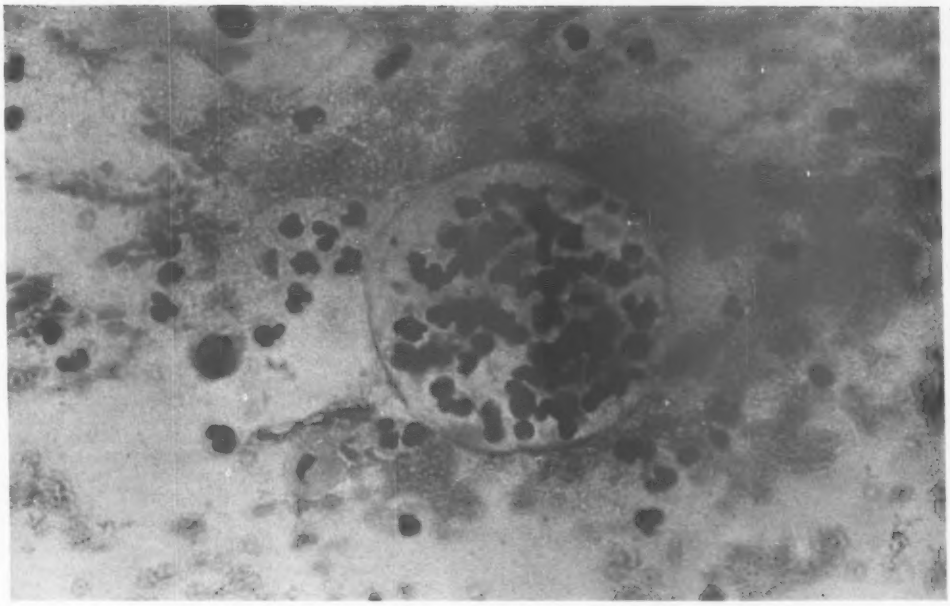


Fig. 11. Polymorphonuclear leukocytes in a cell without a nucleus. The cytoplasmic membrane is easily seen.

Fig. 12. Endocervical carcinoma with glandular and squamoid characteristics.

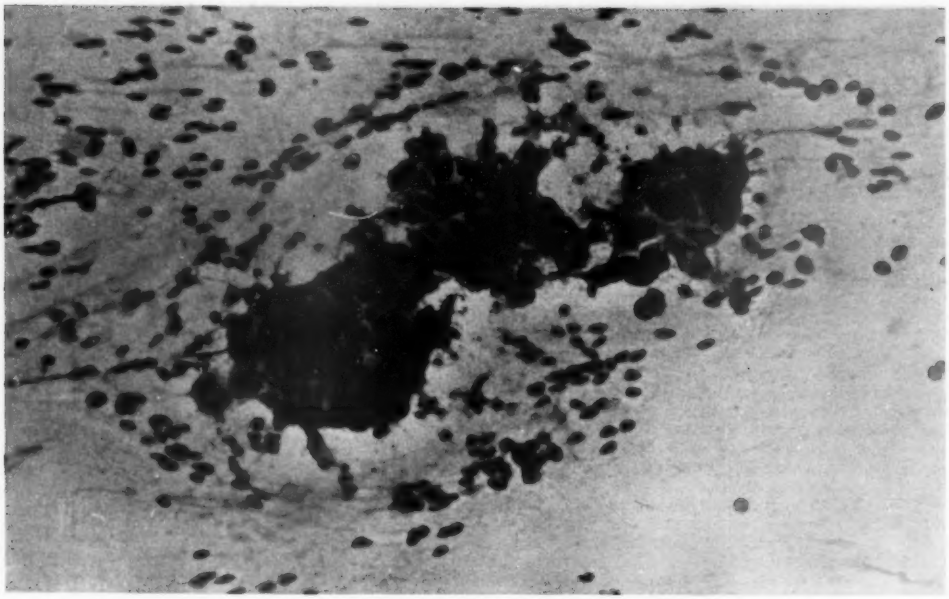


Fig. 13. Endocervical polypoid adenocarcinoma with anomalies.

Fig. 14. Adenocarcinoma cell in metamorphosis (polychromatophilia of the cytoplasm).

B. CORNELIS HOPMAN
Miami, Florida, U.S.A.

Endocervical adenocarcinoma is the most infrequent of the three forms of uterine carcinoma—the squamous cell carcinoma of the cervix, adenocarcinoma of the endocervix, and the adenocarcinoma of the cavum uteri. According to the literature it is one-twentieth as common as the epidermoid cervical variety (1); however, in our laboratory it has occurred even less frequently. It usually begins in the cervical canal and may later be found on the vaginal surface of the cervix or more frequently involving the cervix and adjoining tissue without vaginal extension.

The histologic gland pattern in adenocarcinoma of the cervix may show striking variations. In some cases the glandular epithelium is mostly the thickness of one cell and the carcinoma is diagnosed by the irregular glandular structure, penetration of the cells in the surrounding tissue and the cancerous characteristics of the cells (Fig. 1). In other cases the gland pattern is very different; the glandular epithelium may be many layers thick and may even fill the lumina so completely that the tumor may appear as a solid squamous type of cancer (Fig. 2). The amount of cytoplasm may show great variations. In some endocervical cancers the cytoplasm is restricted to a small rim around the nucleus (Fig. 1). In others there is abundant cytoplasm, often containing vacuoles with mucoid material, giving the characteristics of signet-ring cells (Fig. 2).

The cytologic picture shows the differences in accordance with the histology. The cells are exfoliated in clusters of three to twenty cells, although isolated cells occasionally appear. The nuclei show the known characteristics of cancer: hyperchromasia, coarse clumping of chromatin and prominent single or multiple nucleoli. The cytoplasm may be sparse (Fig. 1a) or abundant (Fig. 2a), but when present it contains mucoid-filled vacuoles of varied size. The cytoplasm stains yellow or reddish with the Papanicolaou staining method.

The cytological differences of endocervical carcinoma and squamous cell carcinoma of the cervix are evident. Due to the external location of the epidermoid cervical cancer (Fig. 3), the cells are exfoliated in masses and occur on the slides in totally irregular patterns, often single but also haphazardly in groups. There is no rule of site or location. The amount of cytoplasm of the cancer cells depends upon the grade of differentiation of the cancer (Fig. 3a).

In endocervical carcinoma the cells are mostly shed in groups, occasionally singly. The color of the cytoplasm in endocervical carcinoma is yellow or reddish due to the amount of mucoid substances. The color of the cytoplasm in squamous cells is blue, red or orange depending upon cornification and differentiation.

The cytoplasm of squamous cell carcinoma does not as a rule contain vacuoles. The cytoplasm of endocervical carcinoma, if present, contains vacuoles. Although form and size of the endocervical carcinoma cells may vary considerably, the variability in squamous carcinoma is much more marked. In squamous carcinoma, squamous cells are present in great numbers; in endocervical carcinoma these cells are scarce. In squamous carcinoma most of the squamous cells show malignant deviations, from slight dyskaryosis to far-advanced malignant characteristics. In endocervical carcinoma the squamous cells present are normal.

Leukocytes in squamous carcinoma are often numerous due to the superficial localization of the lesion giving rise to secondary infection. Although infection is also present in endocervical lesions, the number of leukocytes is proportionately less. Mucus secretions give rise to a pink appearance of the whole slide in endocervical carcinoma, not characteristic of squamous carcinoma.

A summary of the cytology of endocervical adenocarcinoma compared to squamous cell cancer shows the following characteristics:

| | Endocervical Adenocarcinoma | Squamous Carcinoma |
|---|---|---|
| Exfoliation | Clusters of three to 20 cells. Occasionally isolated cells. | Irregular, haphazard. |
| Cytoplasm | Irregular but more equal size, containing vacuoles. Color—yellow or reddish. Occasionally signet-ring cells. | Size varies depending upon differentiation of the cell; no vacuoles as a rule. Color—blue, red or orange depending upon type of cell. No signet-ring cells. |
| Nucleus | Malignant characteristics present in moderate variation. | Malignant characteristics present in extreme variations. |
| Number of squamous cells | Scarce | Numerous |
| Character of squamous cells | Normal | Malignant |
| Number of endocervical cells | Numerous | Not abundant |
| Character of endocervical cells | Malignant | Normal |
| Leukocytes | Moderate in number | Abundant |
| Background | Pink appearance because of abundant mucus production. | No such appearance as a rule; background dirty because of numerous bacteria and leukocytes. |

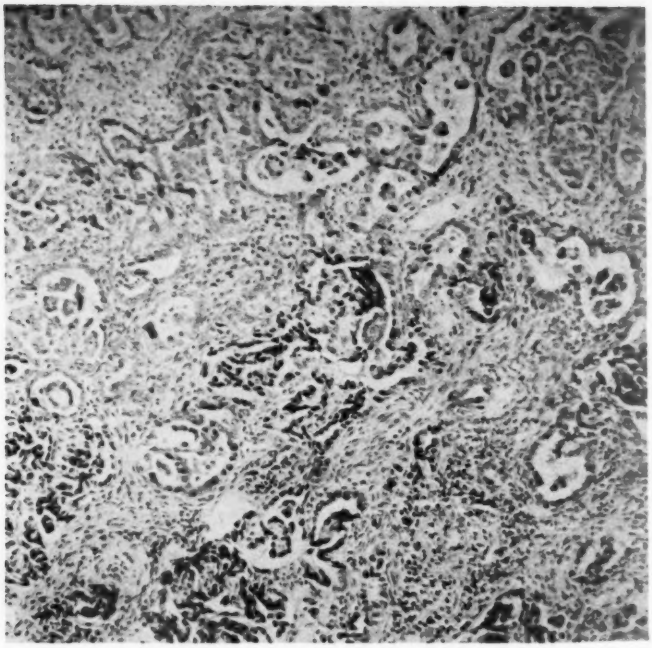


Fig. 1. Histological slide of a patient with endocervical adenocarcinoma. (112X)

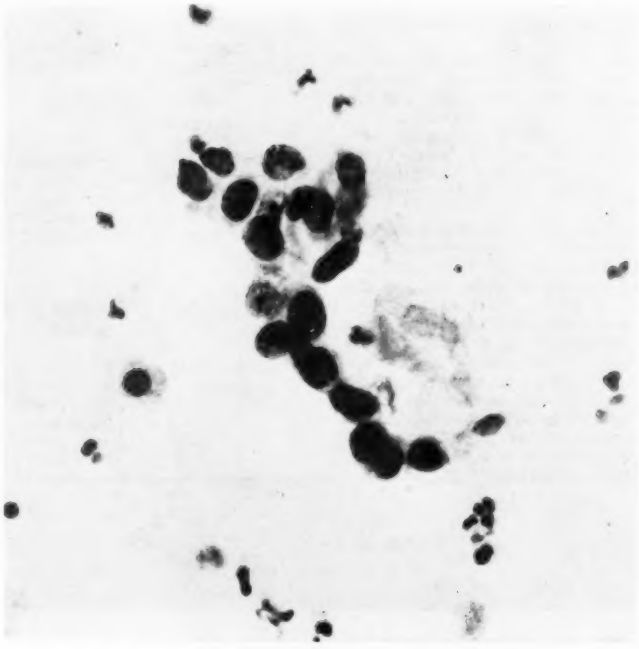


Fig. 1a. Cytological slide of the same patient. (635X)

Fig. 2. Histological slide of a patient with endocervical adenocarcinoma. (108X)

Fig. 2a. Cytological slide of the same patient. (635X)

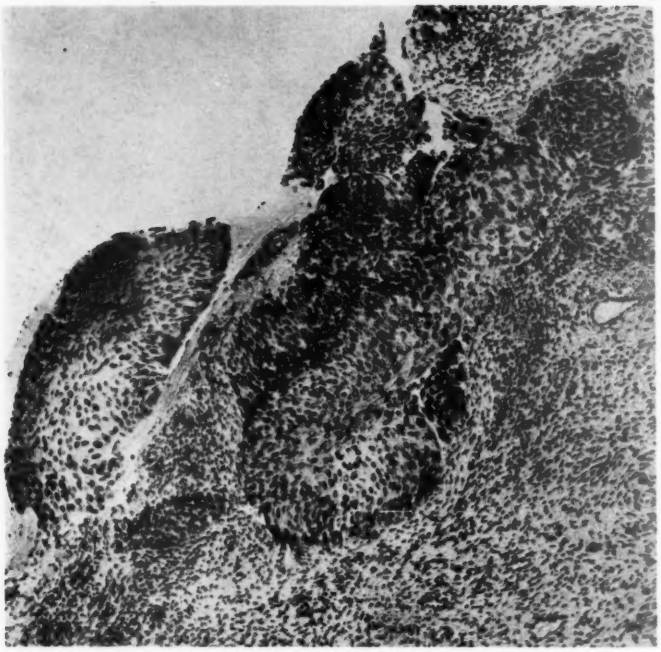


Fig. 3. Histological slide of a patient with squamous carcinoma of the cervix. (102X)

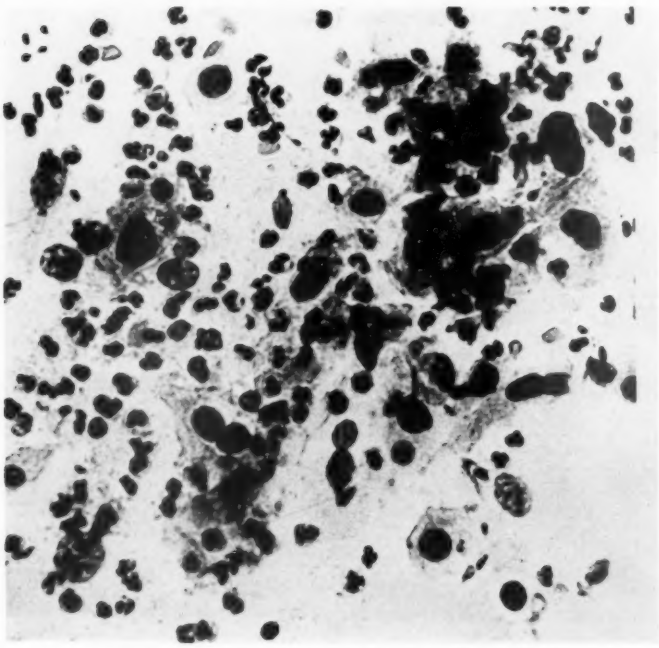


Fig. 3a. Cytological slide of the same patient. (528X)

VIOLETTE M. NUOVO
Paris, France

The cytological differential diagnosis between squamous and adenocarcinoma of the endocervix is sometimes difficult. Therefore we are giving our results and the criteria of both types in this paper. The criteria of malignancy are easily recognized, but are often difficult to differentiate, e.g., 1) the endocervical squamous carcinoma from the ectocervical squamous carcinoma, 2) the endocervical squamous carcinoma from the endocervical adenocarcinoma in cases where the neoplastic cells are poorly differentiated, and 3) the endocervical origin of a typical adenocarcinoma.

Squamous Carcinoma:

The cytological evidence of an endocervical squamous carcinoma does not seem very definite. The diagnosis of malignancy, per se, is easy, and usually so is the identification of the epidermoid nature of the lesion. But this is not so for the identification of the endocervical location of the lesion.

In our material, 18 endocervical squamous carcinomas were cytologically diagnosed as:

typical endocervical squamous carcinoma . . 7
squamous carcinoma without any specific characteristics . . . . . 8
endocervical adenocarcinoma . . . . . 2
carcinoma in situ . . . . . 1

Among the 18 cases, two were carcinoma in situ cytologically diagnoses—one as a cancer in situ and one as a typical endocervical squamous carcinoma.

To diagnose an endocervical squamous carcinoma I depend on the very small size of the neoplastic cells. Very often the cells are poorly differentiated, but sometimes they are well differentiated. The nuclei have regular edges (round or elongated), are extremely hyperchromatic, and contain chromatin clumping.

Adenocarcinoma:

In these cases the malignant cellular pattern is also rather obvious, and often so is its glandular type, but here again the identification of the endocervical location is not easy. In our material of endocervical adenocarcinoma, 24 cases were cytologically diagnosed as follows:

typical endocervical adenocarcinoma . . . . . 11
adenocarcinoma, primary site unknown . . . 6
undifferentiated cancer . . . . . 5
endocervical squamous carcinoma . . . . . 1
no neoplastic cells . . . . . 1

The cytological criteria which I believe to be pathognomic for the endocervical adenocarcinoma are as follows:

1. very large, free nuclei with irregular borders,
2. isolated cells or cells in groups but without overlapping,
3. rather pale nuclei with clumps of chromatin, and
4. often several prominent nucleoli.

The results are summarized in the adjoining table.

## DISCUSSION

EMMERICH VON HAAM, Columbus, Ohio, U. S. A.:

I agree with de Brux and Dupré-Froment on the great difficulty of recognizing cytologically the papillary type adenocarcinoma of the cervix. The double epidermoid and columnar carcinoma always produces abundant malignant squamous cells and is usually recognized cytologically as a malignancy of the squamous cell type. The malignant columnar cells are easily misinterpreted as undifferentiated squamous cells. I have not observed the difference in the color of the cytoplasm in endocervical and ectocervical carcinoma cells as described by Hopman.

OLLE KJELLGREN, Gothenburg, Sweden:

I agree with the above authors on the essential points but would like to point out the decreased tendency of exfoliation in endocervical adenocarcinoma. In a previous investigation it was found that these tumors were

Table I
Endocervical Carcinoma

| Histological Findings | Cytological Findings | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | Typical for endocervical squamous carcinoma | Squamous carcinoma with no characteristics as to site | Undifferentiated, but typically endocervical carcinoma | Adenocarcinoma with no characteristics as to site | Typical for endocervical adenocarcinoma | Carcinoma in situ | |
| Squamous carcinoma: | | | | | | | |
| invasive | 6 | 8 | 0 | 0 | 2 | 0 | 18 |
| in situ | 1 | 0 | 0 | 0 | 0 | 1 | |
| Adenocarcinoma | 1 | 0 | 5 | 6 | 11 | 0 | 23 + 1 false negative |

significantly more difficult to diagnose by means of cytology than the squamous cell carcinomas of the ectocervix.

Bibliography

1. Kjellgren, O.: Acta Obst. & Gynec. Scandinav. 34: 249, 1955.

RAIMUND KRIMMENAU, Dresden, Germany:

Endocervical adenocarcinomas are rare occurrences. It is known to the histologist that adenocarcinomas in certain cases need hardly deviate cellularly from the epithelia of the topical glands. On the other hand, extremely polymorphic cytologic patterns also occur. Cytologically one can, therefore, find polymorphous cells (which cannot be differentiated from the squamoid carcinoma cells) up to almost normal appearing cells. The desquamative tendency is usually also far less in the adenomatous lesion. The adenocarcinomas of the ectocervix observed by us were sometimes already suspected by means of cytology. Inversely, cytologically suspected adenocarcinomas sometimes turned out to be histologically undifferentiated squamous cell carcinomas (or carcinomas in situ).

We like to recognize the following criteria:

1. Surprisingly many adenomatous cells, which at the time of smear preparation were not exfoliated for physiological reasons (menstrual period, glandular hyperplasia, endometritis, polyps, corporal carcinomas may cause difficulties in the differential diagnosis).
2. Predominantly stripped-nuclear elements with anisokaryotic but hardly polymorphous nuclei with moderate chromatin content and sometimes prominent nucleoli.
3. Still recognizable histological groupings such as papillary patterns. We could not find vacuolization and leukocytic phagocytosis occurring with remarkable frequency.

The differential diagnosis with benign conditions of adenomatous character (especially inflammatory metaplastic lesions), and similarly, the differentiation from undifferentiated squamous lesions, appear to us not always possible by means of cytology only.

ALEXANDER MEISELS, México, D. F., México:

There seems to be agreement that:

1. Endocervical adenocarcinoma presents several specific cytological characteristics, which are sufficient in many cases to establish a correct diagnosis by cytological means only,
2. Endocervical adenocarcinoma is readily distinguished from squamous carcinoma,
3. Endocervical adenocarcinoma may be differentiated, although with more difficulty, from endometrial adenocarcinoma, by cytological means.

The presence of very large, clumped, malignant nuclei, surrounded by scanty, vacuolated cytoplasm, in a relatively clean field, and often accompanied by clusters of hyperplastic endocervical cells, is significant evidence for the existence of an endocervical adenocarcinoma.

The endocervical site of the adenocarcinoma is shown not only through the specific morphology of the malignant cells, but also through the presence, in many cases, of groups of hyperplastic endocervical cells. These cells are also frequently found co-existing with squamous carcinoma of the endocervix or with endocervical in-situ carcinoma. They probably represent epithelial changes in the area surrounding the carcinoma similar in significance to the changes often found in the ectocervical epithelium near squamous carcinomas.

GUILLERMO TERZANO, Buenos Aires, Argentina:

The above authors present a convincing description of cytology of endocervical adenocarcinoma and clearly differentiate the cells desquamated from adenocarcinomas and squamous cell carcinomas.

As for morphological characteristics generally accepted as positive signs of malignancy, as mentioned, there should be added some particular features such as those related to the appearance of the foamy and vacuolized cytoplasm in cell clusters or in isolated cells, which could be considered as almost specific and as a sign that may lead towards the diagnosis of adenocarcinoma. Anisokaryosis, usually more prominent than in cases of adenocarcinoma of the endometrium, also may help in the diagnosis. We try not to mistake macrophagia with pseudo-phagocytosis or the inclusion of leukocytes, cellular debris, etc., within large vacuoles, so often observed associated with inflammatory processes.

ERICA WACHTEL, London, England, U. K.:

The discrepancies in the description of the criteria of cervical adenocarcinoma as stated by the three main speakers are bewildering. De Brux stressed the absence of nuclear characteristics usually postulated as essential in the diagnosis of malignancy, whereas Hopman emphasizes the presence of typical malignant-appearing nuclei and Violette Nuovo holds a position somewhere between the two.

From my own experience I fully support Hopman's views. I would not dare to diagnose malignant disease in the absence of malignant-appearing nuclei, and endocervical adenocarcinoma has proved no exception. I regret that none of the three main speakers have disclosed how their smears were prepared. The state of preservation of endocervical cells is better in endocervical aspiration films than in posterior fornix smears; the staining reaction and the clarity of fields are greatly influenced by the amount of mucus and leukocytes, etc. Smear patterns can, therefore, only be compared if identical methods are employed.

I have had, so far, no difficulties in the recognition of malignant disease as such in cases of endocervical carcinoma using mainly posterior fornix aspiration smears, but difficulties have been experienced, at times, in distinguishing, or trying to, between a very poorly differentiated squamous cancer and adenocarcinoma. However, I meet often with the common problem of differentiating between endocervical and endometrial adenocarcinoma. In view of the often considerable histological difficulties in deciding whether an adenocarcinoma present in curettings arose from the endocervix or the endometrium I do not think such an attempt at differential diagnosis on cytological grounds on exfoliated cells is reliable.

## CLOSING REMARKS

B. CORNELIS HOPMAN:

The tendency to decreased exfoliation as mentioned by Olle Kjellgren and Raimund Krimmenau may account for the smaller amount of malignant cells observed in endocervical cancer as compared with the abundance of cancer cells often encountered in squamous cervical cancer.

I agree with Emmerich von Haam, Raimund

Krimmenau and Erica Wachtel that endocervical cancer cells may be difficult to differentiate from undifferentiated squamous cells. In that case neighboring squamous cells may help out to make the diagnosis. In undifferentiated squamous cell cancer most of the squamous cells show malignant deviations varying from dyakaryotic to far advanced malignant properties. In endocervical cancer the squamous cells present are normal.

Guillermo Terzano rightly stresses the significance of the vacuolated cytoplasm as a differential characteristic.

VIOLETTE M. NUOVO:

To Kjellgren: Except for one case, we always had a very important exfoliation of neoplastic cells in cases of endocervical adenocarcinoma.

To Erica Wachtel: I want to make very clear that the cells found on the smears of endocervical adenocarcinoma cases reported had typical malignant nuclei. I probably failed to give a proper description of these nuclei.

For each case I receive two smears:

a vaginal smear (posterior fornix aspiration)
a cervical smear (mainly taken with Ayre spatula).

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

# CAN ONE DISTINGUISH BY MEANS OF CYTOLOGY THE ENDOCERVICAL AND ENDOMETRIAL ADENOCARCINOMA?

B. CORNELIS HOPMAN*
Miami, Florida, U.S.A.

Although more common than adenocarcinoma of the cervix, adenocarcinoma of the endometrium occurs less frequently than epidermoid cervical carcinoma—only one-sixth or one-seventh as frequently. It occurs between fifty and sixty years of age, approximately ten years later than epidermoid cancer. Adenocarcinoma of the endometrium and endocervix, both being glandular lesions, show less striking differences between themselves than when compared to squamous carcinoma.

Endocervical cells are larger than endometrial cells. They show a distinct honeycomb appearance in transverse sections and a columnar arrangement in longitudinal section. The cell border is distinct and mucus or cilia protrude from their free edge. They have a characteristic triangular shape with a basal nucleus.

Malignant endometrial cells vary in size from four to 50 $\mu$ in diameter (1) and are usually encountered in irregularly distributed groups from three to 20 cells; occasionally they are single. There may be only a small rim of cytoplasm or the cytoplasm may reach approximately 10 $\mu$ in thickness. It is irregularly stained, usually includes many vacuoles and occasionally encloses degenerating leukocytes. Nuclei vary in size from four to 40 $\mu$ and are made up of very irregularly stained and unevenly distributed coarse granular chromatin. One or more nucleoli are often seen (Fig. 1a).

The histologic structure of adenocarcinoma of the endometrium has a characteristic pattern. The glands show a disorderly arrangement, with an increase in number and atypical distribution. Now and then the glands are so close together that only a scant amount of stroma is seen. The nuclei of the cells show hyperchromsia, irregular content of chromatin and variations in form and size. The epithelium is often stratified many cell layers thick and may grow so intensely that the gland lumina are solidly filled (Fig. 1).

Although the histological cell pattern is easily distinguishable from adenocarcinoma of the cervix, the cytological picture does not show striking differences. Endometrial cancer cells are usually smaller than endocervical cancer cells. Often they contain less cytoplasm and a smaller nucleus, although there are also types of endocervical cancer cells showing very little cytoplasm. Both contain vacuoles, but those of the endometrial cancer cells are larger, compared to the size of the cell, than the vacuoles of endocervical cancer cells. The cytoplasm of endocervical cancer cells contains more mucoid substances and is yellowish or reddish compared to the bluish-red rim of cytoplasm of the endometrial cancer cells. Both show fewer leukocytes in the cytological background than does the squamous carcinoma. In many cases it will be difficult to distinguish endometrial from endocervical carcinoma by means of cytology.

Of great importance for differentiation is the use of a precision measurement under the microscope as practised by placing a screw micrometer in the eye piece of the microscope (2). In using such an instrument (Leitz screw micrometer) and 500 X magnification (40X for objective and 12-1/2X for ocular) the following measurements are made:

Endocervical cells:

| Cell | Nucleus |
|---|---|
| Up to 4-5 intervals, sometimes fading cytoplasm | Mostly more than 1 and up to 2-1/2 intervals |

Endometrial cells:

| Cell | Nucleus |
|---|---|
| 1 interval, sometimes fading cytoplasm | Mostly 1/2-3/4 up to 1 interval |

Endocervical cancer cells now and then show a signet-ring structure. Endometrial cancer cells do not.

*This work was supported by a grant from the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

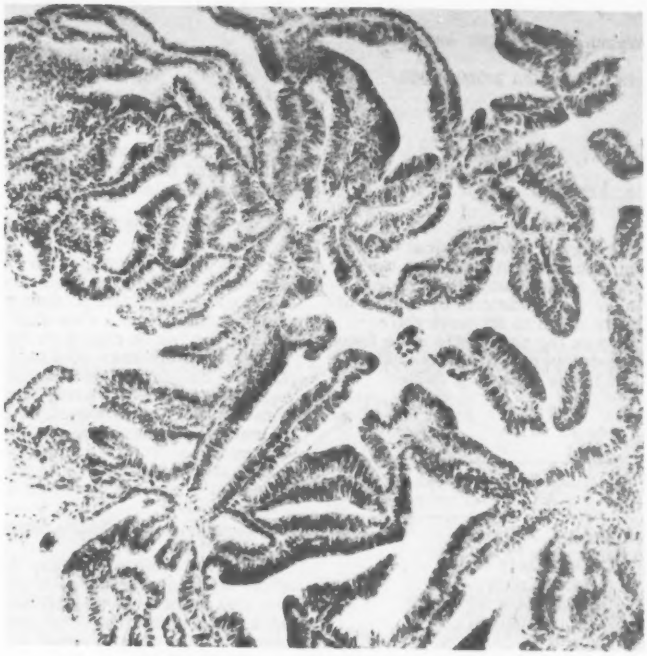


Fig. 1. Histological slide of a patient with adenocarcinoma of the uterus.

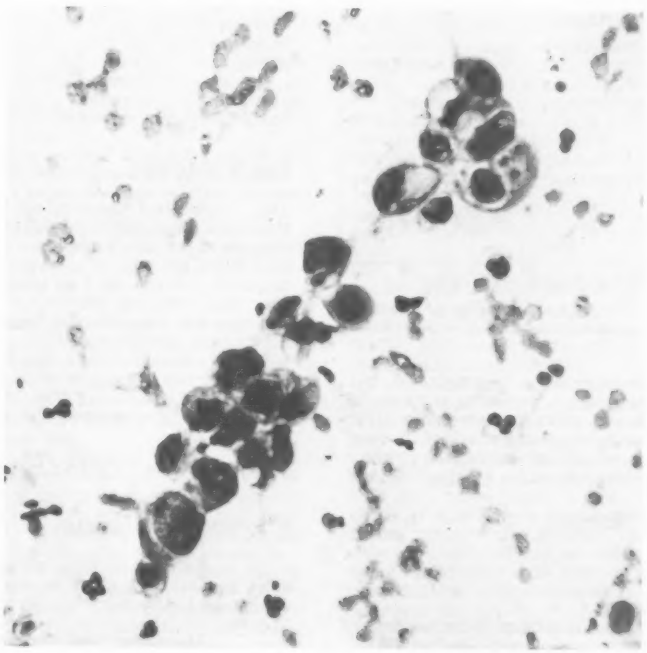


Fig. 1a. Cytological slide of the same patient.

Bibliography

1. Hopman, B. C. and Werch, S. C.: Obst. and Gync. 2:267, 1958.
2. Boschann, H. W.: Acta Cytologica 2:520, 1958.

## VIOLETTE M. NUOVO
### Paris, France

In the section "Cytology of Endocervical Carcinoma" it has already been reported that, although the diagnosis of adenocarcinoma is rather easy, the endocervical site is very difficult for me to identify by means of cytology. Nevertheless, there seem to be more differences than similarities between the neoplastic cells found in cases of endometrial and endocervical adenocarcinomas. This is summarized in the following Table:

Table I

| Endometrial Adenocarcinoma | Endocervical Adenocarcinoma |
|---|---|
| **Nucleus** | |
| Rather small | Extremely large |
| Very hyperchromatic | Rather pale |
| Slightly irregular chromatin | Extremely irregular chromatin |
| Anisokaryosis in the same group | Anisokaryosis in the same group |
| Irregular nuclear borders | Irregular nuclear borders |
| Scarce nucleoli | Presence of one or several prominent nucleoli |
| **Cytoplasm** | |
| Vacuolated | Non-existent or very small, rather homogeneous fragments |
| Sometimes intra-cellular polymorphonuclear leukocytes | No intra-cellular polymorphonuclear leukocytes |
| **Distribution** | |
| Rarely isolated | Often isolated |
| In overlapping groups | When in groups, side by side |
| **Presence of dysplastic squamous cells** | |
| None | Rather often |

This enumeration of criteria is schematic, but it seems that the differences are noticeable. In cases of endocervical adenocarcinoma where we were only sure of the presence of an adenocarcinoma but not of its primary site, we found in almost all instances that it was not a case of endometrial carcinoma.

## DISCUSSION

**JEAN DE BRUX, Paris, France:**

In our opinion, the multiplicity of the aspects of endocervical adenocarcinoma, and especially the often striking identity between the endometrial carcinomas and the endocervical adenocarcinomas, permit the differentiation between the two localizations only if there are involved:

a. very well differentiated carcinomas, recalling an endocervical hyperplasia with cells of eosinophilic cytoplasm, or
b. elements of Gartnerian type.

Furthermore, examples of cannibalism are as often found in the endometrial carcinomas as in the endocervical carcinomas.

**ALEXANDER MEISELS, Mexico, D. F., Mexico:**

Both main speakers have pointed out enough differences between the cells arising from an endocervical adenocarcinoma and an endometrial adenocarcinoma to support the point of view that a correct diagnosis of the site of the lesion can often be done by cytologic means. In addition, one may also take into consideration the fact that the cells from an endocervical adenocarcinoma are usually much better preserved in the vaginal or cervical smears than the endometrial cells. Also, in cases of endometrial adenocarcinoma, blood pyocytes and cellular degeneration are frequently observed. If separate endocervical and endometrial smears are obtained, the differential diagnosis becomes even easier.

**GUILLERMO TERZANO, Buenos Aires, Argentina:**

The morphological differences between endocervical and endometrial cells presented by Smolka (1) and now the presentations of Hopman and Violette Nuovo give us a clear idea of both the ease and difficulties of the interpretations of both types of cells.

It is not extremely difficult to make a diagnosis of adenocarcinoma, but the endocervical localization of the tumor (known after the histopathological report) sometimes comes as an unexpected surprise for the cytologist.

This diagnosis is not impossible, but a careful study of the whole smear is necessary.

Bibliography

1. Smolka, H.: Acta Cytologica 2:515, 1958.

**ERICA WACHTEL, London, England, U. K.:**

While I congratulate Hopman and Violette Nuovo on their ability to differentiate between adenocarcinoma of the endometrium and of the endocervix, I confess that I am unable to do so. I did not find that the variation in cell size was an adequate criterion of distinction; in my opinion the size of the individual cancer cell is related to the type and degree of differentiation of the tumor (e.g., papillary, solid, mucus secreting, etc., adenocarcinoma) rather than to the site of its origin. Likewise, the arrangement of tumor cells in clusters or isolated did not, in our laboratory, appear to be a distinguishing feature between the two sites of origin.

## CLOSING REMARKS

**B. CORNELIS HOPMAN:**

As Jean de Brux points out endocervical cancer cells occasionally show an eosinophilic cytoplasm as compared to the more bluish cytoplasm in endometrial cancer.

Alexander Meisels rightly stresses the fact that for the differential diagnosis it may be important to make separate endocervical and endometrial smears.

# INCIDENCE AND CLINICAL COURSE OF ENDOCERVICAL ADENOCARCINOMA

DIETRICH BUTTENBERG AND
PETER STOLL
Heidelberg, Germany

The endocervical adenocarcinoma is defined by its adenomatous structure and its origin from the glandular epithelium of the cervical canal; however, it may arise not only within the cervical canal but also within the region of the external os, especially in cases of an ectropion of the cervical glands, or it may arise within the cervical wall from mesonephric vestiges. The different varieties represent a special problem not only by their histogenetic origin but also by their clinical course and confront the clinician with special consideration in regard to diagnosis and treatment.

It is generally accepted that adenocarcinoma of the uterine cervix is far less common than epidermoid carcinoma of this region. The percentage is given as low as 2.7% by Healey and as high as 13.5% by Warren, with an average of about 4% (Limburg and Thomsen, Norris). In our material of nearly 2000 very thoroughly investigated cases of cervical carcinoma we have found the distribution of the different types as follows:

85% epidermoid carcinoma (highly mature, moderately mature, and immature)
8% clear cell carcinoma
6% adenocarcinoma
1% special types, such as carcinoma of the Gartnerian duct, carcinosarcoma, melanocarocarcinoma, etc.
6% carcinoma in situ (Stage 0)

The average age of our patients with endocervical adenocarcinoma is 52 years, a little higher than that of patients with epidermoid carcinoma (48 years); on the other hand it is reported that this type of cancer can be found already in very young children, the youngest being a 7 month old infant reported by Bumm. It can be supposed that within the small group of young patients with cervical adenocarcinoma the dysontogenetic origin of the tumor plays an important role in the early development. This is especially true for the adenocarcinoma arising from remnants of the Gartnerian duct.

Distribution of age in patients with endocervical adenocarcinoma

| | |
|---|---|
| 0-20 years | 1.5% |
| 20-30 years | 1.5% |
| 30-40 years | 15.6% |
| 40-50 years | 29.5% |
| 50-60 years | 29.1% |
| 60-70 years | 18.0% |
| 70 and more | 5.4% |

When adenocarcinoma develops at the external os or within the adjoining area of cervical ectropion, it may present itself as a ulcerative or as a papillary lesion, and the diagnosis of adenocarcinoma can only be made microscopically. When it develops within the cervical canal, it represents an ulcerative necrotic area within the field of invasion and destruction, or builds a papillary growth, which also very soon undergoes necrotic changes. The surface of the ectocervix may appear entirely normal by gross inspection, but usually during colposcopy or close inspection with a magnifying glass one can see blood-traces within the cervical mucus. Palpation often reveals the wall of the cervix thickened with tumor, hardened and lumpy. After digital examination, the cervical secretion shows more blood than during the first inspection. Taking the cervical smear is of great diagnostic value and should always be done, if in a clinically suspicious case one does not prefer to immediately take some tissue with a sharp spoon for definitive histological examination.

Not very seldom one sees adenomatous hyperplasia of the cervical glands, sometimes with cystic pattern, but in this case the columnar epithelium forms a single layer of uniform, high, secreting cells of the characteristic "picket"-type (Novak), and the differential diagnosis against malignant growth seems simple. We have only seen a few cases of adenomatous hyperplasia of the cervical glands with tendency towards papillary structures, mitoses and several layers of columnar cells, where we had difficulties deciding whether or not this should be declared as benign. Here we sometimes have a borderline to the highly differentiated type of mucoid adenocarcinoma, in which the very well differentiated single cells apparently have the mucin-producing capacity of the normal columnar cervical epithelium. Here only the proportion of mesenchymal and epithelial structure helps for correct differentiation. If there are only

very small filaments of mensenchymal tissue, which besides this shows hyalinization of the ground-substance, the diagnosis of malignancy seems correct. The term "adenoma malignum" seems confusing for this pattern, because the recurrence of the tumor after local removal and the possibility of metastases verify that this kind of tumor is carcinoma.

The system of gradation proposed by Broders can be used for classification of the tumors, where at one side definite dedifferentiation of many cells as shown by nuclear and cytoplasmic characteristics is seen in an over-all picture of very mild glandular atypia with adenomatous structure, and on the other side the degree of immaturity and mitotic activity of the single cells is combined with very marked atypia of the glandular structure and fields of solid tumor growth resembling an epidermoid carcinoma.

Limburg and Thomsen in their report on 60 cases have formed 3 groups:

| | Limburg and Thomsen | U. of Heidelberg |
|---|---|---|
| highly mature to moderately mature: | 34 | 25 |
| immature | 14 | 25 |
| special forms | 12 | 13 (Carcinoma micropapillare, gelatinous adenocarcinoma, carcinoma of mesonephric origin) |

The type of adenoacanthoma is included in the special forms. The most important question arises with the consideration as to whether the type of tumor has something to do with radiosensitivity and cure-rate.

In 1953 G. Döderlein reported in his review on adenocarcinoma of the cervix an over-all cure rate of 25% (4). This was confirmed in 1954 on 31 cases by Baker, Brack and Dickson (2), and in 1956 with 23% on 28 cases of adenoacanthoma from the Mayo Hospital by Fricke, Loule and Craig (8). On the other hand, the results of Hepler, Dockerty and Randall with a cure rate of 40% in 164 cases are nearly as good as the results in epipermoid carcinoma (10). Our own results during the years from 1935 to 1953 (Heidelberg) are, with an over-all cure rate of 37.1% in 105 cases, below the results in epidermoid cancer and this is also the impression of Brandstetter and Kratochwil of the II. Universitaets-Frauenklinik of Vienna, who during the years from 1934 to 1953 in 60 cases have found an over-all cure rate of only 26.7%. The best results ever reached with radiotherapy alone and reported in the literature are those from the I. Univ. Frauenklinik of Munich (Bickenbach and Ries). They have reached an over-all cure rate of 43.9% in 193 cases during the time from 1940 to 1952 after improving the therapy since 1940. The 187 cases reported by Kottmeier of Stockholm (13), Sweden with a cure rate of 50.8% do not include cases of the International Classification Stage IV and cannot be compared with the above mentioned cases. Taking only the very best results of the last years, in general, the over-all cure-rate of endocervical adenocarcinoma is not as great a success as that of epidermoid carcinoma and remains about 5-10% beneath the average. This is true for radiotherapy but also for elective therapy. This fact was discussed in the literature extensively. First it was supposed that the beginning within the cervical canal or in the cervical wall with no or only very insignificant symptoms would cause a long latent period (von Franque, Nilsson) and at the time of diagnosis the tumor would already have spread out into the parametrical lymphatic vessels and lymph nodes. It was felt that the dissemination of adenocarcinoma of the cervix in the lymphatic route would start earlier than in epidermoid cancer. Novak reported one case in which the cervical lesion measured only a few millimeters, while nests of carcinoma were already seen in the parametrium far below the surface. Also Döderlein, Nilsson and Schrimpf have seen very early dissemination in the lymphatic route and also early metastases in other organs. This is also true in 8 of our 105 cases, in which metastases to the vagina, vulva and ovaries, but also to other distant situations did exist. Abundant evidence of lymphatic dispersion is often seen in areas adjoining the lesion, which might still be covered by normal mucosa. The lymphatic dissemination into the parametric tissue cannot be found as easily by palpation as the involvement of the parametrium in cases of epidermoid carcinoma; therefore, the classification of the tumor by the clinician might be State I or Stage II, while the extension of the tumor is already far more. This makes a correct classification much more difficult than in the case of epidermoid carcinoma.

On the other hand, most authors are convinced that the radiosensitivity of endocervical adenocarcinoma in general is less than that of epidermoid carcinoma (Adler, Brandstetter and Kratochvil, G. Döderlein, Feldweg, von Franque, Hepler and Dockerty and Randall, Kehrer, Kamniker, Lacassagne, Regaud and from our Department Runge and Zeitz); however, Eymer, Ries and Kottmeier with their great experience in radiotherapy could not prove that there is real radioresistency in cases of endocervical carcinoma. Eymer, as well as Wintz, Haupt and Wittenbeck used to give a dosage which is 25% higher than the dosage used in the treatment of epidermoid carcinoma. With radium x-ray therapy alone the following excellent results were reported:

| | Stage I | Stage II |
|---|---|---|
| Kottmeier | 68.1% | 57.7% (Stockholm) |
| Ries | 67.2% | 46.5% (Munich) |

We could not reach the same good results with radiotherapy alone in our cases and prefer, therefore, a more elective treatment as do Bradstetter and Kratochvil, Doderlein, Hepler, Dockerty and Randall.

| | Stage I | Stage II |
|---|---|---|
| Surgery and x-ray | 75% | 60% |
| Radium and x-ray alone | 60.9% | 34.2% |

It is difficult to compare the findings in the different institutions because the lack of a definite standard in the histological type of tumor. Some authors are convinced that cervical adenoacanthoma (Fricke, Loule and Craigh) as well as gelatinous carcinoma cervicitis (Limburg and Thomsen) cannot be influenced by actinotherapy as well as the more or less mature usual type of adenocarcinoma cervicitis. In regard to the carcinoma of the Gartnerian duct, Schmitt has raised the question whether we should define this tumor as malignant or whether it is more a benign variety, while Stoll and Buttenberg are convinced that for this type of tumor the term carcinoma is correct.

Summarizing we might say that endocervical adenocarcinoma reveals a special problem not only in histogenesis and histological structure, but also in regard to diagnosis and therapy. Early dissemination on the lymphatic route, not detectable by gynecological palpation, leads to a better classification (Stages I or II) than is really present. Early metastases to other organs, also to distant organs, occur. Part of the cases, especially those with very high differentiation and extreme mucin production, exhibit a relatively poorer radiosensitivity. Diagnosis can be delayed when the tumor starts within the cervical canal; therefore, endocervical smears play an important role in the diagnostic procedure. Diagnosis by

exfoliated cells alone can be very difficult in a case of highly differentiated tumor. Even the histological diagnosis can be difficult occasionally and borderline cases to simple adenoma of the cervical glands to adenoma of the Gartnerian remnants may occur.

## Bibliography


1. Adler, L.: Zbl. Gyn. 673, 1916.
2. Baker, H. W., Brack, C. B. and Dickson, R. J.: Obst. & Gynec. 4:464, 1954.
3. Brandstetter, F. and Kratochwil, A.: Krebsarzt 14: 251, 1959.
4. Döderlein, G.: Zbl. Gyn. 1529, 1953. Arch. Gyn. 506, 1953.
5. Eymer, H.: Arch. Gyn. 156:268, 1934.
6. Feldweg, H.: Z. Geburtsh. 111:1, 1935. Strahlenth. 46:110, 1953.
7. Von Franque, O.: Strahlenth. 21:187, 1926.
8. Fricke, R. E., Loule, E. H. and Craig, R. M.: J. Amer. Geriatr. Soc. 4:113, 1956.
9. Haupt, W.: Z. Geburtsh. 103:103, 1932. Strahlenth. 46:311, 1932.
10. Hepler, K. T., Dockerty, M. B. and Randall, L. M.: Am. J. Obst. 63:800, 1952.
11. Kamniker, H.: Zbl. Gyn. 457, 1932.
12. Katz, H.: Arch. Gyn., Kongr. Sdb. 134, 1927.
13. Kottmeier, H. L.: Gynaecologia 134:243, 1952.
14. LaCassagne, A.: Strahlenth. 33:91, 1929.
15. Limburg, H. and Thomsen, K.: Das Adenocarcinom des Collum uteri. Stuttgart, 1949, Georg Thieme.
16. Von Mikulicz-Radecky, F.: Münch. Med. Wschr. 47: 1996, 1931; Zbl. Gyn. 13, 1934.
17. Nilsson, F.: Acta Radiol. 4:283, 1933; 16:219, 1935.
18. Norris, O.: Am. J. of Cancer, 21:653.
19. Novak, E.: Gynecological and Obstetrical Pathology. Philadelphia, 1947, W. B. Saunders.
20. Philipp, E.: Zbl. Gyn., 931, 1932.
21. Regaud, C.: Strahlenth. 31:671, 1929.
22. Ries, J.: Med. Klin. 30:1346, 1959.
23. Runge, H. and Zeitz, H.: Geburtsh. und Krauenhk. 10:875, 1956.
24. Schmitt, A.: Geburtsh. und Frauenhk. 6:502, 1951.
25. Stoll, P. and Buttenberg, D.: Geburtsh. und Frauenhk. 8:698, 1955.
26. Wintz, H.: Münch. Med. Wschr. 46:1931, 1935.


## NO CLOSING REMARKS

# CYTO- AND HISTOMORPHOLOGY OF CARCINOMA OF THE GARTNERIAN DUCT

DIETRICH BUTTENBERG AND
PETER STOLL
Heidelberg, Germany

The vestigial Wolffian duct proceeds in the mesosalpinx as the Gartnerian duct, extending downward at the lateral wall of the uterus, sometimes included in the uterine musculature, forming a loop within the cervical wall and then going down along the lateral wall of the vagina, occasionally extending into the vulva region. Remnants of the duct can be found in various portions of its course and may show cystic distention or adenomatous proliferation, forming rather large cysts within the mesosalpinx or the vaginal wall. Within the cervical wall remnants of the Gartnerian duct can build adenoma or real adenocarcinoma. In the benign cases the lining epithelium presents many variations. Usually a single layer of cylindrical cells, occasionally showing cilia, can be found, which often, and especially in the case of cyst formation, is flattened, but also differentiates into stratified squamous epithelium. Also in the malignant cases the variability of the morphological cell pattern is obvious, forming an adenomatous pattern as well as a solid pattern. The Gartnerian duct was primarily described by H. T. Gartner, a Danish anatomist, in 1822 in the genital organs of a cow. Follin (1850), Beigel and Kolliker (1879) were the first who described remnants of the Wolffian duct in men. In 1883 Dohrn in his intensive studies of the subject came to the conclusion that in men remnants of the Gartnerian duct are rarely seen after the second half of the embryonic development, that only parts of the duct in most cases are patent, with higher frequency of the right than of the left side. Our knowledge of the normal and pathological anatomy of the Gartnerian duct is based on the findings of Robert Meyer, who in the years 1903-1906 already described two cases of carcinoma of the duct. He very thoroughly studied the incidence of persistency in the fetus and in the adult and found remnants in 100% of the fetuses of 2-3 months of age, in 33% of the fetuses of 4-6 months of age, in 16% of the fetuses of 7-9 months of age, in 12% of the newborn infants and in 24% of the adults. Also, J. Sakuraoko (1937) found a frequency of about 20%, confirmed by Limburg and Thomsen, while J. Huffman (1948) could find remnants of the Gartnerian duct only 5 times in 1192 extirpated uteri and S. A. Wolf (1950) in 1413 cases only once. It can be estimated that 20% is the highest percentage of remnants of the Gartnerian duct which can be found in the adult woman. Supposing that cervical remnants of the duct have to be present in order to form an adenomatous or adenocarcinomatous growth within the cervical wall, the frequency of Gartnerian tumor might be even higher than reported in the world literature. Before 1955 twenty-seven cases of carcinoma of the Gartnerian duct were known, since that time 8 new cases were published. Two additional cases were seen by us in 1957 and 1958. Table I shows that of those 37 cases two are located in the vaginal wall (Szendy Wahlen and Gymning) and one at the border of the isthmus into the corpus uteri (Stoll and Buttenberg). The rarity of the diagnosis might be very well seen in the following difficulties:

1. The diagnosis of a Gartnerian carcinoma is apparent only in such cases in which the uterus is removed and the tumor is found to be localized within the lateral wall of the cervix uteri, and, if possible, normal remnants of the duct are found within the surrounding area by thorough examination of the specimen.
2. If the tumor growth is already advanced, the carcinoma has broken through the ectocervical surface and destruction and ulceration occur, the diagnosis can only be made by the typical morphological picture, described later on, and this might falsely be interpreted as real adenocarcinoma of the cervix.
3. If only biopsy material is available and the patient is treated by radiotherapy, the diagnosis is extremely difficult, especially in such cases, in which biopsy did miss the area, in which the typical picture is present.

Therefore, it can be supposed that the frequency of Gartnerian carcinoma is higher than generally expected.

If one follows the opinion that the diagnosis can be made very well in early cases of Gartnerian carcinoma, but might be missed in advanced cases, it is obvious that the over-all cure rate of this early carcinoma is better than the cure rate of the cervical adenocarcinoma in general. This has led Schmitt to the conclusion that the behavior of a Gartnerian tumor would not rectify the term of malignancy, but only of "relative malignancy." He found that the reported cases did show very good operability, did not show signs of metastases to the surroundings and did exhibit very good curability as compared

Table I

| Authors | Reference | No. of Cases |
|---|---|---|
| Meyer, R. | Virch. Arch. 174, 1903 | 1 |
| Meyer, R. | Z. Geburtsch. 58:528, 1906 | 1 |
| Wagner, G. A. | Zbl. Gyn. 21, 1929 | 1 |
| Daneel, H. | Arch. Gyn. 159:395, 1935 | 1 |
| Rochstroh, H. | Z. Geburtsh. 112:95, 1936 | 1 |
| v. Breitenberg, H. | Arch. Gyn. 171:539, 1941 | 1 |
| Bernstein, A. | Mschr. Geburtsh. 117:81, 1944 | 2 |
| Gosch, J. | Z. Geburtsh. 129:103, 1948 | 1 |
| Huffmann, J. | Am. J. Obstetr. 56:23, 1948 | 1 |
| Huber, H. | Z. Geburtsh. 129:139, 1948 | 1 |
| Huber, H. | Z. Geburtsh. 131:1, 1949 | 1 |
| Limburg, H. and K. Thomsen | Das Adenokarzinom des Collum uteri. Stuttgart, 1949, Thieme. | 3 |
| Lindemann, G. | Arch. Geschwulstforsch. 2:215, 1950 | 1 |
| Stoll, P. | Geburtsh. u. Frauenhk. 10: 219, 1950 | 1 |
| Baltzer, H. | Geburtsh. u. Frauenhk. 6: 499, 1951 | 1 |
| Schmidt, A. | Geburtsh. u. Frauenhk. 6: 502, 1951 | 1 |
| Willems, W. | Arch. Gyn. 179:489, 1951 | 1 |
| Wagner, H. | Zbl. Path., 1952 | 1 |
| Stoll, P. and D. Buttenberg | Geburtsh. u. Frauenhk. 15: 698, 1955 | 6 |
| Niendorf, F. | Geburtsh. u. Frauenhk. 15: 692, 1955 | 3 |
| Wahlen, T. u. J. Gyming | Acta obstetr. scand. 34: 120, 1955 | 1 |
| Szendi, B. | Wien. Klin. Wschr. 875, 1956 | 1 |
| Freese, U. | Geburtsh. u. Frauenhk. 16: 526, 1956 | 1 |
| Kniepkamp, G. | Zbl. Gyn. 80:575, 1958 | 1 |
| Caranagh, D. | Obstet. and Gyn. 13:285, 1959 | 1 |
| Stoll, P. and D. Buttenberg | New cases | 2 |
| | | 37 |

with the ordinary cancer of the cervix. None of the reported tumors has, according to Schmitt, killed the patient. Also, Niendorf came in 1955 to the same opinion and gave the suggestion to eliminate the term "Carcinoma of the Gartnerian duct" and to replace this by "Tumor of the Gartnerian duct," in order to avoid the expression of malignancy. Szendy followed this suggestion in 1956. We are fully aware that one has to distinguish between carcinoma and adenoma of the Gartnerian remnants, as Baltzer already has pointed out in his excellent study of the problem. The picture of Baltzer gives the very best instructions of this question. However, nobody can doubt that real cancer of the Gartnerian remnants occurs and will kill the patient, if treatment is insufficient or delayed.

Of nine cases observed in our Department, five died of carcinoma. In three of those cases we could prove recurrence histologically after surgical radical removement of the tumor. One patient died during the surgical procedure. Another patient lives 10 years after therapy. The remaining two cases observed 1957 and 1958 respectively are still living and are asymptomatic. These last two cases are published herewith:

1957: patient 47 years of age, partus: none, abortus: none, conservative myomectomy 1939. Discharge for five months. Tumor of the left lateral cervical wall. Biopsy revealed the diagnosis: squamous epithelial cell carcinoma. Clinical grouping: cervical carcinoma Stage I. A Wertheim radical hysterectomy was performed. In the surgical specimen the definite histological diagnosis was: carcinoma of the Gartnerian duct, located at the left lateral wall of the uterine cervix.

1958: patient 18 years of age, partus: none, abortus: none, patient is virgo. Enlargement of the left lateral wall of the cervix uteri with ulceration. Biopsy: squamous cell carcinoma. A Wertheim radical hysterectomy was performed. In the histological specimen the diagnosis of a Gartnerian duct—carcinoma was obvious.

In conclusion it is felt: out of the 37 published cases the definite diagnosis of Gartnerian Carcinoma was almost without exception made from the surgical specimen. Therefore, those cases did apparently present good operability. It is our opinion that biopsies alone are often insufficient for obtaining proper classification of the tumor as a carcinoma of the Gartnerian duct. In the large group of advanced cervical carcinomas treated radiologically and diagnosed before by biopsy only might be hidden a certain number of carcinomas of the Gartnerian duct.

In regard to the diagnosis cytology plays an enormous role, as it does generally in early cases of cervical carcinoma; however, the role of cytology is even greater, because the location of the tumor within the lateral wall of the cervix and the early communication to the cervical canal leads to the exfoliation into the cervical canal. One of our cases, which did not show any clinical symptoms at all, reveals on routine cytology (endocervical smear) the presence of small atypical cells with very dark and polymorphous nuclei, resembling the cell type seen in tubal carcinoma. Diagnosis of malignancy was made cytologically and the following biopsy proved the presence of carcinoma of a special, less differentiated cell type. Within the surgical specimen, if enough material is available for the histological diagnosis, one mostly finds two very characteristic patterns of malignant tissue:

a. Adenomatous proliferation with a lining epithelium, which might be cylindric or cuboidal, occasionally exhibiting papillomatous growth. The single cells usually are of the same size and form with only slight variability. Cystic dilatation is often present. Within the cysts a homogenous material is found, which on mucin reaction is negative. The epithelium can change into a cuboidal form which builds several layers. The general pattern may also change into a more tubular type. Transition into solid areas is observed.
b. The tumor is growing in coarse solid cones, consisting of cells which may exhibit great variability depending on the grade of differentiation. There is occasionally seen abundant cytoplasm, resembling the so-called clear-cell-type, while in other areas the solid parts are formed by little round epithelial cells containing round hyperchromatic nuclei with only a small margin of cytoplasm.

Within the cytological preparation the cell pattern may vary widely, but cells of an undifferentiated type with dark round nuclei and a small margin of cytoplasm

rarely are missed.

On biopsy the diagnosis of adenocarcinoma or of squamous carcinoma is usually made; in fact, the real diagnosis of Gartnerian carcinoma on the first slide is seldom.

The clinical appearance is that of a cervical carcinoma with enlargement of the lateral wall of the cervical canal and that of ulceration in case of advanced carcinoma. From the surgical specimen diagnosis of Gartnerian duct carcinoma can be made by the typical histological picture, which changes in different areas of the tumor, by location within the cervical wall, and last, not least, by proving remnants of the Gartnerian duct, not destructed by carcinoma, below or above of the lesion.

NO CLOSING REMARKS

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

*Symposium C*

# TRAINING OF THE CYTOTECHNOLOGIST

## EDUCATIONAL PREREQUISITES FOR CYTOTECHNOLOGISTS

WERNER BICKENBACH AND
HANS-JÜRGEN SOOST
Munich, Germany

Regarding the pre-education for cytotechnologists one should request the same conditions as those demanded for the general medical technician. In Germany this means six years of "Oberschule" or "Gymnasium" or so-called "Mittelschule" with a final examination.

The starting age should not be less than sixteen. Thus training will not be finished earlier than eighteen years of age. In our opinion, boys and girls of lower age groups do not always have the mental maturity which is required for independent work in professional life.

Further training depends entirely on given possibilities. If there is a separate school for cytotechnologists which, of course, has to be connected with a cytological laboratory, all the general medical and cytological training, theory and practice, can be fulfilled in one place. This is not yet possible in any country except perhaps in some places in the United States.

If it is impossible to visit a school for cytotechnologists, general medical education might consist of a visit to a teaching institute for medical technicians with a final state examination for this profession. But it might also be an education as a physician's aide. This should also be performed at a school acknowledged by the state, and should last one year. We especially want to emphasize that we do not think the education as a medical technician a firm condition for future activity as a cytotechnologist. However, necessary as a general medical education is, one should nevertheless avoid burdening the cytotechnologist with too many teaching-subjects. One should especially think of this because the profession of a cytotechnologist is rare and apparently every country is short of trainees.

During the general preparation time, at least at the beginning of the essential cytological training the future cytotechnologist should at least learn rudimentary typewriting and shorthand.

Selecting the students for cytotechnology one has to refer to mental ability and character. The work requires a fairly good intelligence and especially conscientiousness and feeling for liability, since the cytotechnologist will work not only as an aide in the techniques and organization, but also as a helper to the physician by prescreening smears, and prescreening a great number of preparations requires a great deal of patience.

BIRGITTA FREDRIKSON
Gothenburg, Sweden

Considering that Sweden is a small country where exfoliative cytology still is not accepted in all quarters, it is extremely important that the cytotechnologists be well qualified for their work. It seems that the training for histological technicians is basically a good

place to begin. There he will obtain theoretical training in anatomy, histology, pathology, chemistry, photography and microphotography, statistics and experimental work with animals contemporaneously with two months work in some of the clinical laboratories. During this period of training he will become acquainted with laboratory work of all kinds which will be a good background for studies in exfoliative cytology. He will also be well prepared in handling the different scientific problems with which he will be working. Since all literature on exfoliative cytology is, for us, in a foreign language, the cytotechnologists should be able to read at least English and German.

With this background the student has all the theoretical qualifications for being a good cytotechnologist as long as he has the personal qualifications which in this kind of work are extremely important. Exactitude, carefulness and patience are the main prerequisites for a good cytotechnologist.

## EDMUND SCHÜLLER AND MARGARETE PEUTSCH

### Vienna, Austria

In Austria the desirable requirements for pretechnical training are graduation from high school and the diploma for "medizinisch-technischer Assistent." These technicians have three years of courses at the Medical Faculty of the University and are trained in medical chemistry, anatomy, histology and bacteriology, both theoretically and practically. After completion of the training period the candidates take an examination for qualifying as a certified medical technologist (medizinisch-technischer Assistent).

It would be desirable for future cytotechnologists to do practical work in a general medical laboratory for several months after the examination before starting their cytology training.

## DISCUSSION

**RACHEL R. ACHENBACH, Salem, Massachusetts, U.S.A.:**

Although I agree that each of the programs described would provide a sound background for any student cytotechnologist, the division between the educational prerequisites and the theoretical training in cytology seems to be extremely hazy. This can, perhaps, be attributed to variations in local training facilities.

I believe that the minimal educational prerequisites could be safely limited to training in basic cytology, gross and microscopic anatomy and physiology, particularly that relating to the female reproductive system. Many high school graduates have received such training, and, if maturity were not such an important factor, many of them would prove to be desirable students. I do not feel that a student engaged in a training program which provides a carefully planned theoretical education in exfoliative cytology, will find himself seriously handicapped if he lacks previous experience in chemistry, bacteriology, hematology, or pathology. On the other hand, if the student is to be expected to muddle through a few textbooks and some demonstration slides pretty much on his own, he definitely needs previous medical training in any field which includes some gross and microscopic pathology, and preferably some laboratory experience.

**MARIA S. BLANCO de del CAMPO, Palo Alto, California, U.S.A.:**

I agree with Bickenbach and Soost as well as Schüller and Margarete Peutsch that the cytotechnology candidate benefits from the experience of working as a medical technician, nurse or secretary. The prerequisites of the American Society of Clinical Pathologists, —MT, for cytotechnology—to which I subscribe, correspond for the most part to those in Sweden as outlined by Birgitta Fredrickson. However, I do not feel exposure to courses in pathology, microphotography, statistics and animal care are essential. Personality traits such as endurance and keen visual discrimination as well as those listed by Bickenbach and Soost are also very important in the selection of trainees.

**ALICE D. CLARK, Chicago, Illinois, U.S.A.:**

It was interesting to me that Bickenbach and Soost as well as Birgitta Fredrikson pointed out the necessity of certain prerequisites of personality and mentality besides those of education. In guiding a person in or into a professional career in cytotechnology, these qualifications might be minimized or overlooked, qualifications which are essential to a high-level cytotechnologist.

There is difficulty in drawing up an overall picture of educational prerequisites from countries where the educational systems differ. In Sweden, Birgitta Fredrikson suggests starting by following the program for training of histological technicians . . ." contemporaneously with two months work in some of the clinical laboratories." Does this mean that the period of training is only two months for cytotechnologists? If so, what training has taken place before this? Is this the only prerequisite for cytotechnologists?

In Germany and Austria, the prerequisites include general medical and related subjects and seem to take a period of two to three years followed by a certifying examination. Bickenbach and Soost mention to "avoid burdening the cytotechnologists with too many teaching subjects" during this period. Could they give some examples of these "teaching subjects"?

In all of these countries, is there any standardization of training for cytotechnology controlled by a governing board and terminated with a certifying examination, as there seems to be for medical technologists?

**ELIZABETH H. GRAY, Ottawa, Ontario, Canada:**

All of the authors seem to be fairly well agreed that a thorough general medical technician's training is a desirable prerequisite for the cytotechnologist. Having had this training and many years of medical laboratory experience myself, I admit its value, but not its necessity. Hematology, and perhaps bacteriology, proved to be a good basis for the study of cytology. Theoretical training in anatomy and histology are most essential. It is possible to take such extension courses at some universities. A college diploma is, unfortunately, no more a guarantee of personal integrity and mental maturity than a marriage certificate is of emotional stability. I believe more attention should be focused on opening the field of cytology to intelligent, mature persons well beyond their teens, who may have only a good basic education, but who will stay in the field for many years: the widow with children to support, the nurse with tired feet, the paraplegic, the heart case. Cytology is a difficult discipline at best and experience is the only teacher. We are defeating our own ends by encouraging teen-aged women into this field, only to run into competition with early marriage and the stork.

PATRICIA KELLY, San Francisco, California, U.S.A.:

The courses in theoretical training mentioned above certainly are desirable and the personal characteristics most essential for a good cytotechnologist. An additional indispensable qualification is a genuine interest in exfoliative cytology. If the candidate does not have the desired educational requirements, but does have personal integrity, responsibility and a keen interest in cytology you may be sure that study in related fields will follow as a natural consequence. In my opinion a candidate with these three qualifications should be given an opportunity to prove her ability in the field.

IRENA KOPROWSKA, Philadelphia, Pennsylvania, U.S.A.:

Among three opinions given by authors from Germany, Sweden, and Austria respectively, the latter two agree that prerequisites for cytotechnologists should include college courses and training with or without certification in medical technology. The first pair of authors are setting somewhat flexible standards, based upon realistic considerations of the difficulty in finding prospective cytotechnologists.

A similar difference of opinions concerning educational prerequisites seems to exist in the United States of America among those concerned with teaching cytotechnology. In our School of Cytotechnology, first preference for admission is given to college graduates who are registered medical technologists. Former instruction in histology and chemistry are considered most important and experience in hematology is highly desirable. One wonders, however, if requirements should not be allowed to vary in different areas according to local conditions, as suggested by Bickenbach and Soost. With an increase in the availability of candidates for training and of good training centers, an adequate number of cytotechnologists will presumably become available. Consequently, the progressive restandardization of prerequisites at a higher requirement level should be carried out periodically.

The first two authors also stress certain personal characteristics as important prerequisites. Among such undeniable qualities as good intelligence, conscientiousness, responsibility, exactitude and carefulness (each of which was mentioned once), patience was pointed out as essential by both authors. Bickenbach and Soost also mention age as an important consideration. We tend to favor candidates well in their twenties or older, in preference to "at least 18 years old" applicants. In our experience, the restlessness of teens is rarely compatible with a great deal of patience, a need which is not unduly stressed.

RAIMUND KRIMMENAU, Dresden, Germany:

According to conditions prevailing in Europe—as also all other reporters describe them—a cytotechnologist can only develop himself practically out of the so-called "medizinisch-technischen Assistenten." For cytological work, however, still more interest, diligence and intellectual independence must be required. Up to the present only a few medical technicians have become interested in such a development of studies and the acceptance of greater responsibility, since no equivalent remuneration can be offered. It should be of interest to be informed whether or not in the United States of America the cytotechnologist is immediately employed in cytology without a general medical-technical education, and whether or not he is paid equally well or better than the other laboratory workers.

JOSÉ R. DEL SOL, Madrid, Spain:

Our cytotechnologists in Spain are all female. It may be that since most of them are nurses, they have become interested in this type of work through daily contack in the hospital and clinics.

We divide the educational prerequisites into a minimum and optimum.

Minimum: "Bachillerato Superior." This degree is equivalent to the American high school plus two years of college. "Bachillerato Elemental" plus a degree in nursing. An American high school diploma is equivalent to the "Bachillerato Elemental." To obtain the degree in nursing, three years of practical and theoretical studies are required.

Optimum: A degree in Medicine or Pharmacy.

Minimum prerequisites plus a working knowledge of two foreign languages, preferably one of them being English, are desirable. The time required for the formation of a capable cytotechnologist with minimum educational prerequisites is reduced to one-half in the group with optimum prerequisites.

CHARLOTTE M. STREET, New York, New York, U.S.A.:

I agree that the educational prerequisites for cytotechnologists should be comparable to those for medical or histology technicians. However, of the subjects listed for the latter by Birgitta Fredrikson, photography, microphotography, statistics and experimental work with animals, while they greatly enhance the value of the cytotechnologist, particularly in teaching or research laboratories, seem unnecessary for routine screening.

A course in medical technology, as advocated by Schüller and Margarete Peutsch, prior to more specialized training in cytotechnology has certain advantages. It provides valuable training and experience in general laboratory routine and microscopy. In small hospitals where the practice of exfoliative cytology is still somewhat limited, the cytotechnologist is often expected to perform other procedures as well. However, with the present shortage of cytotechnologists, an extra year's training in medical technology seems superfluous.

The prerequisites as established in the United States of America by the Registry of Medical Technologists (ASCP), which probably correspond in most respects to those mentioned by the speakers, should be the minimum requirements. They are two years of college, including twelve semester or eighteen quarter hours of biological sciences. The most necessary basic courses are histology, anatomy and physiology. Bacteriology, parasitology, embryology and chemistry are also helpful. The ideal preparation would include four years of college because of the broader education and the greater maturity of the graduate. This, however, is perhaps impractical in most instances.

DOROTHY B. WELLER, Providence, Rhode Island, U.S.A.:

It is generally agreed that a prospective cytology student must have patience, be intelligent and conscientious. Beyond this, it would seem that the opportunity for ideal preparation for the study of cytology varies greatly from Europe to the United States. Lacking an opportunity to study science on the college level, it would be better for the student to have any available experience in medical techniques or laboratory procedures than to have no pre-medical training at all. However, a minimum of two years of college, including courses in chemistry, anatomy, histology, embryology, bacteriology, and other allied sciences, provides a sounder background for further training in cytology. It may be hoped that in the future when there is not such a scarcity of trained cytotechnologists as now exists, the minimum educational requirement will be raised to four years of college spent in the study of science courses.

The broader the knowledge of the student in gen-

eral science, the more rapidly she will progress in an understanding of cytology, with fewer problems for the instructor in the training program.

## CLOSING REMARKS

**WERNER BICKENBACH and HANS-JÜRGEN SOOST:**

May we answer the questions of Alice Clark as follows:

We believe that we could dispense with the following teaching subjects which actually belong to the general training program of the medical technologists: x-ray diagnostic procedures, x-ray therapy procedures, physical therapy, various special clinical tests such as blood sugar, etc., electrophoresis and electrocardiographic procedures. The following subjects could be treated in a rather shortened form: bacteriology, hematology, numerous clinical tests.

We consider the concentration of the teaching program on subjects which concern cytological evaluation important, because the future cytotechnologist must learn many subjects which may not be so useful for the medical technologist working in a general clinical laboratory. There is no State Board for cytotechnologists in Germany. We have one for the general medical technologists.

**BIRGITTA FREDRIKSON:**

To Alice Clark I want to emphasize that the training for medical technologists in Sweden takes two years and is completed by a certifying examination. The first year consists of theoretical studies in the subjects mentioned and at the same time practical work in different laboratories, two months in each of them. The second year is entirely devoted to practical training, and now the future cytotechnologist can start studying cytology. As there are still very few cytology laboratories in Sweden, the training is individual, given by the doctor and the senior cytotechnologist. I might add that all senior cytotechnologists in Sweden have had some period of cytology training abroad, mainly in the U. S. A.

I agree with Charlotte Street that for routine screening the subjects mentioned might be unnecessary, but as the cytotechnologist, at least in small laboratories, has to assist in scientific work, I have found knowledge in these subjects of great value.

I agree with Elizabeth Gray that cytology is not a field for teen-aged women; in Sweden we are also troubled by the stork.

**EDMUND SCHÜLLER and MARGARETE PEUTSCH:**

It is of great advantage to select the cytotechnologist from the ranks of the general medical technologist. These individuals have had a good basic training in anatomy, histology, physiology, etc., and one can tell from their past performance whether they are better fit for the microscopical work rather than for the chemical-technical procedures. In addition, the fact that the general medical technology examination was passed is evidence of a certain degree of intelligence and ability to learn as well. The training program is usually geared to the type of material concerning teaching in the particular laboratory. We begin our program with gynecological cytology. If the future cytotechnologist has obtained a general knowledge in the specialty to make him a reliable screener, we then begin to teach him the cytological peculiarities of other body regions. Unfortunately, we lose our pretty young girls due to marriage. If possible, one should try to train those individuals whom one may not expect to lose shortly to marriage. On the other hand, one cannot state that personnel consisting merely of invalids or matrons would provide ideal climate and working conditions.

# SHOULD CYTOTECHNOLOGY BE TAUGHT IN CENTRALIZED SCHOOLS OR IN INDIVIDUAL LABORATORIES?

EDMUND SCHÜLLER AND
MARGARETE PEUTSCH
Vienna, Austria

In our opinion, teaching in individual laboratories is better than in centralized schools. The teacher has more personal contact with the students and soon will notice whether or not the trainee is suited for microscopic work and whether or not he has the necessary qualities for a cytotechnologist, i.e., exactness, diligence, etc. Training in centralized schools may not result in such selection.

MARIA S. BLANCO de del CAMPO
Palo Alto, California, U.S.A.

The teaching of cytotechnology should be undertaken in an institution fulfilling these minimal requirements:

1. A teaching staff consisting of a director, a chief cytotechnologist and an assistant cytotechnologist. The director, preferably a pathologist, ought to be able to devote 10% of his working schedule supervising the training; the chief cytotechnologist and assistant instructor may spend as little as 30% in direct instruction. Visiting physicians should be invited to lecture on principles of their specialties that relate to cytology.
2. A special student room equipped with audiovisual aids such as projectors and a library provided for lectures and discussions.
3. The laboratory receiving no fewer than 1000 specimens per month, and of these at least 10% other than gynecological.
4. Affiliation or close association of the laboratory with a medical organization or hospital which allows the student to collect specimens or to observe the mechanics of specimen collection.

It is difficult to know exactly what distinction between centralized school and individual laboratories is intended in the question. The crucial requirements are an adequate volume of material and a suitable staff for teaching. The attempt has been made above to specify these requirements in greater detail. Where they are met, it does not seem decisively important whether or not the institution is a centralized school or an individual laboratory.

JEAN A. de BRUX
Paris, France

Personally, I believe that nothing can replace the practical work of a laboratory. It is in fact by the daily routine work that the technician will rapidly acquire a sense of his responsibility, and because of this he will direct very special care to his screening, and in the presence of difficulties will ask for an explanation.

I believe also that practical training should be accompanied by the acquisition of theoretical notions, which will not be given immediately but progressively, in such a way that the technician has first had contact with the difficulties of cellular diagnosis.

This, however, is valid only for laboratories in which the novice technician is in contact with a trained cytologist who has decided to train his technician as his co-worker and also with other technicians much more experienced than he is and who have an excellent background in cytology and histology.

Nevertheless, I believe that the presence of good technicians at lectures and at courses for doctors is also desirable.

WERNER BICKENBACH AND
HANS-JÜRGEN SOOST
Munich, Germany

As far as the theoretical part of cytotechnology training is concerned it would doubtless be an advantage

if the training could be received at school, especially the general medical pre-education and also the theoretical cytological knowledge. This would be better since individual training at the laboratory includes the danger that the theoretical training might be neglected or that the laboratory's chief (gynecologist, pathologist) might be biased in a particular branch of work.

The practical training doubtlessly would be better in a laboratory, for we all know that medical technicians who come directly from school have to adjust themselves to their environment before they are able to do independent work.

Also, in cytology there are too many things that cannot be learned by theory alone or by looking at a few typical samples. The efficiency and accuracy in cytodiagnosis depends on sufficient experience. In our opinion, the best solution should be aimed at a compromise in which the theoretical pre-education is performed at school and the practical training is performed individually in a laboratory. Practical training should be given only by those cytological laboratories that are acknowledged as working scientifically and are well organized and see at least 5,000 smears per year.

## DISCUSSION

RACHEL R. ACHENBACH, Salem, Massachusetts, U. S. A.:

All of the authors apparently agree that a school that cannot provide sufficient practice material for the development of the students' diagnostic efficiency is inferior to a teaching laboratory with ample material but which may lack a formal theoretical training program.

I agree with Bickenbach and Soost that perhaps a happy solution to many of our problems might result from a separation of the teaching programs. I think most busy laboratories would welcome a recognition of centralized schools for cytotechnology designed to offer only a formal teaching program. Unlike many laboratories, a school is presumably able to offer a course of lectures by highly competent instructors in different fields. Assuming the availability of good demonstration material, it would be equipped to provide excellent theoretical training in all areas of exfoliative cytology to a maximum number of students in a minimum amount of time. These students could then scatter to complete their microscopic training in any approved, well-organized laboratory, where they would be an asset rather than a time-consuming liability.

I should like to add that for a student in training, a large volume of material cannot substitute for a good, working system for histo-cytologic correlation. His triumphs and his errors must be discovered quickly if the student is to progress rapidly.

J. ERNEST AYRE, Miami, Florida, U. S. A.:

Cytotechnologist training may be undertaken equally well in centralized schools or individual laboratories. The important criteria would seem to be an experienced cytologist or cyto-pathologist as teacher with an adequately large and active volume of cytological material available for instruction and study.

BIRGITTA FREDRIKSON, Gothenburg, Sweden:

I agree that elementary practical training is most favorable with individual instruction in a well-acknowledged laboratory, and as de Brux suggests contemporaneously progressive theoretical training.

To guarantee a well-rounded and standardized training to the advantage of the cytotechnologist as well as the heads of the laboratories, training in a centralized school for some time and the possibility of following the work in some other laboratories after the initial practical laboratory training is recommendable.

MARCEL GAUDEFROY, Lille, Nord, France:

In my opinion, the theoretical and fundamental cytological knowledge should be provided in schools by centralized teaching. The necessary individual practical training of the cytotechnologist will be better obtained during a training of several months (up to one year) under the direct instruction of the teaching staff in an individual laboratory well acknowledged for its scientific standing.

ELIZABETH H. GRAY, Ottawa, Ontario, Canada:

These papers seem to revolve upon the question as to whether or not formal or informal cytologic training is the more desirable. I believe a combination of both is the answer. The initial training, of from four to six months, could best be spent in an acknowledged scientifically organized cytology teaching center, where classes of from twenty to thirty students could be handled. The competition in a group of this size is a valuable stimulus to study on the part of the students and to those teaching to give forth their best efforts. This formal study could then be followed by a period of apprenticeship in a screening laboratory under a pathologist who is qualified in cytology.

H. HUSSLEIN, Vienna, Austria:

The establishment of centralized schools for the education of cytotechnologists seems to be impossible for small countries in the near future. I would tend to believe that the education in the individual laboratories is not only adequate, but also more advantageous from the standpoint of a sound practical training. This is true provided that these individual training laboratories are under the direction of highly qualified cytologists, are fully equipped and would each screen at least 10,000 cases per annum. The theoretical pre-education can be accomplished easily in such an individual laboratory, whereas the practical training will be sounder than in any centralized school.

PATRICIA KELLY, San Francisco, California, U. S. A.:

I share the opinion of de Brux that nothing can replace the practical work in a laboratory. Although theoretical education may be acquired to great advantage before working in a laboratory, I believe the theoretical training must be repeated during the practical training in order to make the practical training meaningful.

To properly fulfill its function, however, a teaching laboratory must be well-staffed with teaching personnel, amply provided with informative cytologic material, and adequately supplied with modern teaching facilities. In my opinion the ideal has been achieved when the students' room is a part of the laboratory proper. In this way the student will become a part of a functioning laboratory and through this association she will better understand routine procedures.

When these conditions are present, and the laboratory is affiliated with a hospital, I agree with Maria Blanco de del Campo, "it does not seem decisively important whether or not the institution is a centralized school or an individual laboratory."

RAIMUND KRIMMENAU, Dresden, Germany:

I agree with the opinion of the main speakers, that the most important part of a cytotechnical training must take place in individual laboratories. The additional theoretical training should be very valuable for the cytotechnologist in training as well as for the instructor. We do not consider a centralized education good without con-

tinuous contact with the actual practice of cytology.

Unfortunately, in this part of the country cytology is still very little propagated, and non-medical people, that is to say so-called "medizinisch-technische Assistentinnen," are only rarely trained in this field.

HANNAH PETERS, Copenhagen, Denmark:

I agree most heartily with de Brux, Schüller and Margarete Peutsch, that individual training in an active cytology laboratory under the personal guidance of the cytologist gives the best results. However, cytology has reached such wide acceptance that training of cytotechnologists on a more routine basis has become necessary in several countries. Here training schedules will have to be set up in a more formal way, offering theoretical as well as practical training. The theoretical part can be taught in courses in the set-up of a school, but I would agree with the above authors that the practical training and experience should be acquired in a cytology laboratory that has active routine work as well as a research program.

LUIS MONTALVO-RUIZ, Madrid, Spain:

I agree with Bickenbach and Soost that the theoretical portion of training should be taught in a specialized center and the practical part could be done in individual laboratories. Nevertheless, the ideal situation would be to have both the practical and theoretical teaching in individual laboratories. In our personal experience we have used both methods. The most important fact is the teaching by a cytologist with extensive experience and a considerable amount of smears available for study. I completely agree with Schüller and Margarete Peutsch.

CHARLOTTE M. STREET, New York, New York, U. S. A.:

I agree with Maria del Campo that cytotechnology can be taught in either centralized schools or in individual laboratories, provided certain requirements are met. Either one must have a professional and technical staff which is well trained in exfoliative cytology and has sufficient time to devote to teaching.

In order to offer both theoretical and practical training, a centralized school must be closely affiliated with a cytology laboratory which can furnish not only diversified, well-documented teaching material, but also a sufficient number of current specimens to provide practical experience in technical procedures and the screening of unknown smears.

On the other hand, an individual laboratory must not be biased in a particular branch of work, a danger mentioned by Bickenbach and Soost. In this respect, I regard the figure of 10% non-gynecological specimens suggested by Maria del Campo as too low for a well-rounded course in exfoliative cytology, considering the fact that specimens from the respiratory, genito-urinary and gastro-intestinal tracts, exudates, etc., must all be derived from that rather small proportion of the whole.

PASCO R. DEL VECCHIO, Bethesda, Maryland, U. S. A.:

I concur with the majority of the participants that practical training is best achieved in the individual laboratory, providing it is well equipped in materials and personnel. Theory is best taught in a school.

A solution might be found in the establishing of an academic course in Exfoliative Cytology in our colleges. The course would be similar to that offered Medical Technologists. It would consist of three years of theory and practical education and one year of practical training in a recognized institution. A Bachelor Degree in Exfoliative Cytology could be awarded on completion of the course.

## CLOSING REMARKS

WERNER BICKENBACH and HANS-JÜRGEN SOOST:

The discussion showed general agreement. It remains to be seen if the general theoretical agreement will be followed by practical work according to the established rules. We should have at least one school of cytotechnology and several well established training laboratories in cytology in each country. These schools could take over the program for each country. If the training could not be performed in a school of cytotechnology, the teaching laboratories should put greater emphasis on the theoretical portion of the training program.

MARIA S. BLANCO de del CAMPO:

I thank the above authors. In regard to the comments by Charlotte Street, I agree that the figure of 10% non-gynecological specimens may not appear ideal yet; I feel it is practical. To date only a few of the qualified training centers may claim a volume larger than 10% of body fluid specimens in their current working load, to request all of them a minimum over this is to add a restriction not essential for the fulfillment of our goals. The knowledge concerning non-malignant, atypical, and malignant cells achieved from the studies of gynecological specimens if the modifications of the epithelium in the various other organs are born in mind. Although I am not in favor of large classes as suggested by Elizabeth Gray, I find del Vecchio's alternate solution attractive.

EDMUND SCHÜLLER and MARGARETE PEUTSCH:

In this country, and probably in most small countries, there is no, and will probably be no school of cytotechnology. Disregarding this fact, we believe that the training in individual well-established and large laboratories seems more advantageous than the training in a specialized school, an opinion which seems to be shared by the majority of the discussants.

# WHICH BOOKS AND MONOGRAPHS ARE SUGGESTED FOR THE CYTOTECHNOLOGIST PRIOR TO AND DURING THE PRACTICAL TRAINING?

MARIA S. BLANCO de del CAMPO
Palo Alto, California, U.S.A.

Although the library of a cytotechnologist may begin with only one book, Papanicolaou's Atlas, the books and monographs listed below will also prove useful, either directly or by supplying valuable information.

Papanicolaou, G. N. and Traut, H.: Diagnosis of Uterine Cancer by the Vaginal Smear. New York, 1947, The Commonwealth Fund.

Papanicolaou, G., Traut, H. and Marchetti: The Epithelia of Women's Reproductive Organs. 1948, The Commonwealth Fund.

*Vincent Memorial Hospital Staff: Cytologic Diagnosis of Cancer. Philadelphia, 1958, W. B. Saunders.

*Gates, O. and Warren, S.: Handbook for the Diagnosis of Cancer of the Uterus by the Use of the Vaginal Smear. Cambridge, Mass., 1950, Harvard University Press.

*Nieburgs, H. E.: Cytologic Technics for Office and Clinic. New York, 1956, Grune and Stratton.

*Novak, E. E.: Gynecologic and Obstetric Pathology. Philadelphia, 1957, W. B. Saunders.

Trussell, R. E.: Trichomonas vaginalis and Trichomoniasis. Springfield, Ill., 1947, C. C. Thomas.

Bernstine, J. B. and Rakoff, A. E.: Vaginal Infections, Infestations and Discharges. New York, 1953, Blakiston.

*De Robertis, E. D. P., Nowinski, W. W. and Saez, F. A.: General Cytology. Philadelphia, 1954, W. B. Saunders.

Cowdry, E. V.: Cancer Cells. Philadelphia, 1955, W. B. Saunders.

*Netter, Frank: The Ciba Collection-Reproductive System. Summit, N. J., 1953, Ciba Pharmaceutical Products.

*Bailey, F. R.: Textbook of Histology. Baltimore, 1958, Williams and Wilkins.

Maximow, A. and Bloom, W.: A Textbook of Histology. Philadelphia, 1957, W. B. Saunders.

Conn, H. J.: Biological Stains. Geneva, N. Y., 1953, Biotechnical Publishers.

Lillie and McClung: Manual of Histotechniques. Washington, D. C., Armed Forces Institute of Pathology.

*Farber, Rosenthal, Alston, Benioff and McGrath: Cytologic Diagnosis of Lung Cancer. Springfield, Ill., 1950, C. C. Thomas.

Mazer and Israel: Diagnosis and Treatment of Menstrual Disorders and Sterility. New York, 1951, Paul Hoeber.

American Cancer Society (Massachusetts Division) Inc.: Cancer: A Manual for Practitioners. Boston, 1950, Committee on Publication.

Dorland, W. A. N.: Illustrated Medical Dictionary. Philadelphia, 1957, W. B. Saunders.

*Cancer Cytology and Cytochemistry, N. Y. Academy of Sciences, Vol. 63, article 6, pp. 1033-1462, 1956.

The laboratory should subscribe at least to the following journals:

*Acta Cytologica
Cancer
Obstetrics, Gynecology and Surgery
Obstetrics and Gynecology

*Highly recommended.

BIRGITTA FREDRIKSON
Gothenburg, Sweden

I suggest to cytotechnicians the following books and monographs prior to and during their practical training:

Vincent Memorial Laboratory Staff: The Cytologic Diagnosis of Cancer. Philadelphia, 1950, W. B. Saunders. The book gives a good description of the cells, both in pictures and text. It is exemplary in disposition and is easy to understand for the untrained cytotechnologist.

Nieburgs, H. E.: Cytologic Technics for Office and Clinic. New York, 1956, Grune & Stratton. This gives a survey of the methods of obtaining material and the set up of laboratories, making it a useful elementary book.

Papanicolaou, G. N.: Atlas of Exfoliative Cytology. Cambridge, Mass., 1954, Harvard University Press. This is an indispensible monograph for the cytotechnologist in her daily work.

Pundel, J. P.: Les Frottis Vaginaux et Cervicaux and Les Frottis Vaginaux Endocriniens. Paris, 1950, Masson. These two books deal with endocrinological cytology.

Smolka, H. and Soost, H.-J.: Grundriss und Atlas der gynakologischen Cytodiagnostik. Stuttgart, 1956, Georg Thieme. This gives a good fundamental introduction to the cytology applied to gynecological endocrinology.

## WARREN R. LANG

### Philadelphia, Pennsylvania, U.S.A.

Cytology cannot be learned from books alone. It takes effort, interest and work in dealing with actual slides in the cytology laboratory. Nor is cytology an isolated discipline. Cytotechnologists are not expected to be physicians with an over-all knowledge of the clinical aspects of the lesions suspected by cytology, the pathological basis, or the therapeutic implications; but they should be indoctrinated with the viewpoint that by their activities they furnish auxiliary information on both benign and malignant changes.

Cytotechnologists must be stimulated by their superiors who should periodically conduct slide reviews with corresponding histopathologic slides. They should be encouraged to read, discuss and question journal articles and books. Preferably they should see how smears are obtained in real patients. In our institution, cytotechnologists-in-training are permitted to work in the Vaginitis and Colposcopic Clinics to see the variants of vaginal and cervical diseases at first hand. They are informed of local hospital and regional cytology meetings and are urged to attend.

We are of the opinion that each laboratory should have its own looseleaf manual for techniques and nomenclature so that intramural uniformity is possible. However, the English language publications most helpful are listed below in alphabetical order. This is not meant to minimize the importance and worth of the many books on cytology written in other tongues.

Ayre, J. E.: Cancer Cytology of the Uterus. New York, 1951, Grune & Stratton. Characterized by excellent printing with many extremely beautiful pictures, this monograph presents some concepts which are not widely accepted. Nevertheless the student can profit from its use.

Bernstine, J. B. and Rakoff, A. E.: Vaginal Infections, Infestations, and Discharges. New York, 1953, Blakiston. Since a great deal of gynecologic cytology relates to vaginal cytology, this monograph is worthwhile in that it presents background and sidelights which serve to orient the student. The book is well written, affording easy reading.

De Allende, I. L. C. and Orias, O.: Cytology of the Human Vagina. New York, 1950, Hoeber. This publication is translated from the Spanish by George W. Corner. It contains relatively few pictures but is more than an adequate summary of the endocrinologic basis of cytology.

Gates, O. and Warren, S.: A Handbook for the Diagnosis of Cancer of the Uterus by the Use of Vaginal Smears. Cambridge, Mass., 1950, Harvard University Press. This handbook is useful primarily because of its 60 clear, black and white plates with adjoining descriptions.

Liu, W.: An Introduction to Gynecological Exfoliative Cytology. A Manual for Cytotechnicians. Springfield, Ill., 1959, C. C. Thomas. Recently published, this book fills a definite and long-standing need for an introductory text at the cytotechnologist's level.

Nieburgs, H. E.: Cytologic Technics for Office and Clinic. New York, 1956, Grune & Stratton. Techniques for all phases of cytology are herein detailed, sufficient to clarify the "how" rather than the "why" of cytology.

Osborn, G. R.: Applied Cytology. Butterworth. An excellent British introductory text of cytology that is probably more meaningful for advanced than beginning students.

Papanicolaou, G. N.: Atlas of Exfoliative Cytology. Cambridge, Mass., 1958, Harvard University Press. This volume is written by Dr. Papanicolaou, the "father" of cytology. Its wealth of material and its unusual organization may be somewhat overwhelming for the initiate but it is a "must" to keep on file in every cytology laboratory.

Vincent Memorial Hospital Staff: The Cytologic Diagnosis of Cancer. Philadelphia, 1950, W. B. Saunders. This particular book, although it might well be revised and brought up to date, is of great value because of its pictures with accompanying diagrams and text. It stresses the normal and then well describes the abnormal.

There are two journals and one transaction which should be on the mailing list of every cytology laboratory:

Acta Cytologica. This is a journal with which cytotechnologists should be well acquainted. The differences of opinion expressed may be disturbing to the beginner but to the advanced student they are undoubtedly provocative.

ISCC-Cytology Newsletter. The ISCC-Cytology Newsletter is a fledgling periodical which so far has dealt mainly with news of the Inter-Society Cytology Council (ISCC) and its activities. Undoubtedly, the scope will be expanded.

Transactions of the meetings of the Inter-Society Cytology Council. These transactions of the meetings are published yearly and present a bird's-eye view of the current status of cytologic thought and research.

## DISCUSSION

JEAN DE BRUX, Paris, France:

I agree completely with Lang, that all the books are good provided that the technician is trained beforehand by a cytologist who is capable of helping him understand the interest of this discipline. The science of cytology is not an isolated study dealing with a jumble of cells of different forms and sizes. It should be integrated with the pathological and biological contexts.

The purpose of the texts, however excellent they may be, is simply to recall and resume with precision that which has already been learned in the laboratory.

PATRICIA KELLY, San Francisco, California, U. S. A.:

The books, monographs and journals suggested above seem to me to be very effective vehicles of information for cytotechnologists. The two that I prefer are Papanicolaou's Atlas and Acta Cytologica. Although I agree completely with Lang that books should not replace the study of smears, I believe too, that educative, well-illustrated literature provides the novice technician with knowledge that she has not had time to acquire through experience.

Three books that I would like to suggest, in addition to those already noted, are:

1. Riley, Gardner M.: Gynecologic Endocrinology. New York, 1959, Hoeber-Harper.
2. Robbins, Stanley: Textbook of Pathology.

Philadelphia, 1957, W. B. Saunders.
3. Ciba Foundation Group No. 3: Cancer of the Cervix. Boston, 1959, Little, Brown and Co.

Lang's suggestion that each laboratory provide a manual for techniques and nomenclature is an excellent one and students find such notebooks of great value. In addition, I would like to suggest a "reprint file." By labeling manila folders to correspond to Papanicolaou's Atlas, worthwhile articles may be filed for future reference.

HOWARD L. RICHARDSON, Seattle, Washington, U. S. A.:

The widespread field application of cytology as a clinical adjunct to the diagnosis of early cancer is still a problem to the pathologist, clinician and technologist. The unexplored problems that still exist make it extremely difficult for any one textbook to answer all of the questions. The individual director or associates may be in disagreement with the present-day texts and thus the technologists entering this field for training will be caught in a crossfire of conflict. In a training program, it is best that the technologist be indoctrinated with the views of the cytopathologist. A standard nomenclature should be adopted; the training period of slide preparations and colored lantern slides should demonstrate the cell types. This period of orientation will take approximately six months. Following this, the technologist should then be encouraged to launch upon individual guided programs of study. The textbooks and monographs as listed by Maria S. Blanco de del Campo and by Lang should be utilized. Discussion periods and critical evaluation of the published reprints will materially aid in the maturation of the cytotechnologist.

IRENA KOPROWSKA, PRIMA VASKELIS and A. DARNELL CORSEY, Philadelphia, Pennsylvania, U. S. A.:

A total of 14 books, including monographs, manuals, symposia and five periodicals were suggested by three different authors. Of these, Nieburgs' "Cytologic Technics for Office and Clinic," and the Vincent Memorial Hospital Group's "Cytologic Diagnosis of Cancer" were the only ones recommended by all three authors. Two of the three authors agreed about four additional publications, namely: Berstine and Rakoff's "Vaginal Infections, Infestations and Discharges," Gates and Warren's "Handbook for the Diagnosis of Cancer of the Uterus by the Use of Vaginal Smear," Papanicolaou's "Atlas of Exfoliative Cytology," and "Acta Cytologica." The remaining textbooks, monographs, manuals and periodicals were recommended by a single participating author alone. This diversity of opinions concerning recommended reading may result from the lack of availability of a truly excellent textbook, lack of agreement about comparative value of different publications, unfamiliarity of instructors with a growing number of cytologic writings and/or any combinations of these factors. It is presumably not the function of discussants to present another list of textbooks, journals and reprints which would better suit their own ideas. A mere comment will be made upon the relative value of having all listed sources of knowledge available, unless a comprehensive guidance in selection of reading matter with ensuing discussions is provided.

The discussants are surprised that authors made no distinction implied by the title between reading suggested prior to and during the practical training. At the Hahnemann Medical College School of Cytotechnology, reading of selected chapters from standard anatomy, histology and pathology textbooks is assigned prior to actual training in microscopic cytology. Students may often alternate reading periods with practical training in technical laboratory procedures. During the initial phase of practical training in actual exfoliative cytology, relatively little reading of cytologic literature is encouraged, emphasis being placed on gaining familiarity with recognition of cells under the microscope. A "Manual for Cytotechnologists," from Cornell University Medical College, and the "Atlas of Exfoliative Cytology" by George N. Papanicolaou, are the most encouraged. When a student's knowledge of cytology progresses adequately, further reading guidance is based upon considerations involving the educational background of the student, his special interests and the general need to develop a comprehensive view of the field.

## CLOSING REMARKS

WARREN R. LANG:

Patricia Kelly's suggestion that each laboratory provide a reprint file of significant articles is an excellent one. I agree with her also that Riley's book should be available as reading material. I concur with Irena Koprowska, Vaskelis and Corsey that reading in standard anatomy, histology and pathology textbooks should be fostered. Primarily, as Richardson states, the cytotechnologist, especially the initiate, must be indoctrinated with the views of the laboratory head to prevent a confusing "cross-fire" of opinion.

The learning process, whether related to cytology or any other discipline, requires active participation from both teacher and student, and I feel that de Brux's statement that the study of cytology "be integrate with the pathological and biological contexts" cannot be forgotten. I repeat my first sentence that "Cytology cannot be learned from books alone." I am delighted, however, that there is so much interest in which books are o value for cytotechnologists.

# PROGRAMS OF PRACTICAL TRAINING FOR CYTOTECHNOLOGISTS

WERNER BICKENBACH AND
HANS-JÜRGEN SOOST
Munich, Germany

The schedule for training ought to be split into two sections: A. general pre-medical education with special regard to further cytological activity and B. special cytological training.

A. General pre-medical education

1. The practical training should begin as an aide in a hospital or in a physician's office. The purpose of this practical work, which has to be performed all day long for three months or half a day for six months, is to acquaint the future cytotechnologist with the institutions of a hospital or physician's office and to achieve a connection with the patient and an understanding of general medical problems. Choosing the branch in which practical training is performed will be decisive, according to the field in which the cytotechnologist will mainly work. For gynecological cytodiagnosis only practical training should be done in gynecological fields; for lung cytology such training should be in a surgical hospital or a hospital for lung diseases.
2. In the course of practical training the student should assist the physician in taking cytological smears and should take cytological smears by himself on occasion. This is the only way to become acquainted with the difficulties of the technique in taking smears. Thus he can judge his own mistakes as a result of any error in taking the smears.
3. The student cytotechnologist should learn about the most important diseases he will be dealing with by the activities in the hospital or in the physician's office. He should also watch the progress of these diseases and learn something about therapeutic methods.
4. We think it a further advantage if the student practices some simple laboratory tests during his practical activity in the hospital, such as urinalysis, blood counts, and differential blood counts.
5. Students who wish to work mainly on gynecological cytodiagnosis should have an opportunity during practical training and after proper theoretical preparation to assist with the colposcopic examinations and to look at colposcopic pictures.

B. Special cytological training

In special cytological training the first weeks and months must be reserved for learning routine technique and organization. Staining of smears, including the preparation of staining material, watching the origin of mistakes caused by different staining reactions, classifying material, filing, and contacting physicians, belong to the general routine in the organization of any laboratory.

If the student is sufficiently accustomed to the above activities, he may start practicing the judgment of smears. The following procedure proved helpful to us in the individual training of physicians or cytotechnologists in the field of gynecological cytodiagnosis.

1. The student first becomes acquainted with basic cytodiagnosis by comparing the literature. We use the book by Smolka and Soost: "Grundriss und Atlas der gynäkologischen Cytodiagnostik," George Thieme, Stuttgart. (If the education is received at school, the study of literature can be replaced partly by theoretical teaching.)
2. Using a collection of selected smears, the student looks at typical preparations under the microscope.
3. For further practice the student reviews older preparations, comparing the findings. (Every smear is kept on file by us for control purposes. We have a duplicate slide of every case.)
4. The final step is the pre-screening of smears. This means that the student looks at the smears which are not judged, puts down his findings and compares them later with the findings of the cytologist. If possible the student should be present when the cytologist judges the preparations. Thus differences or obscurities in findings can be discussed immediately. A special double-

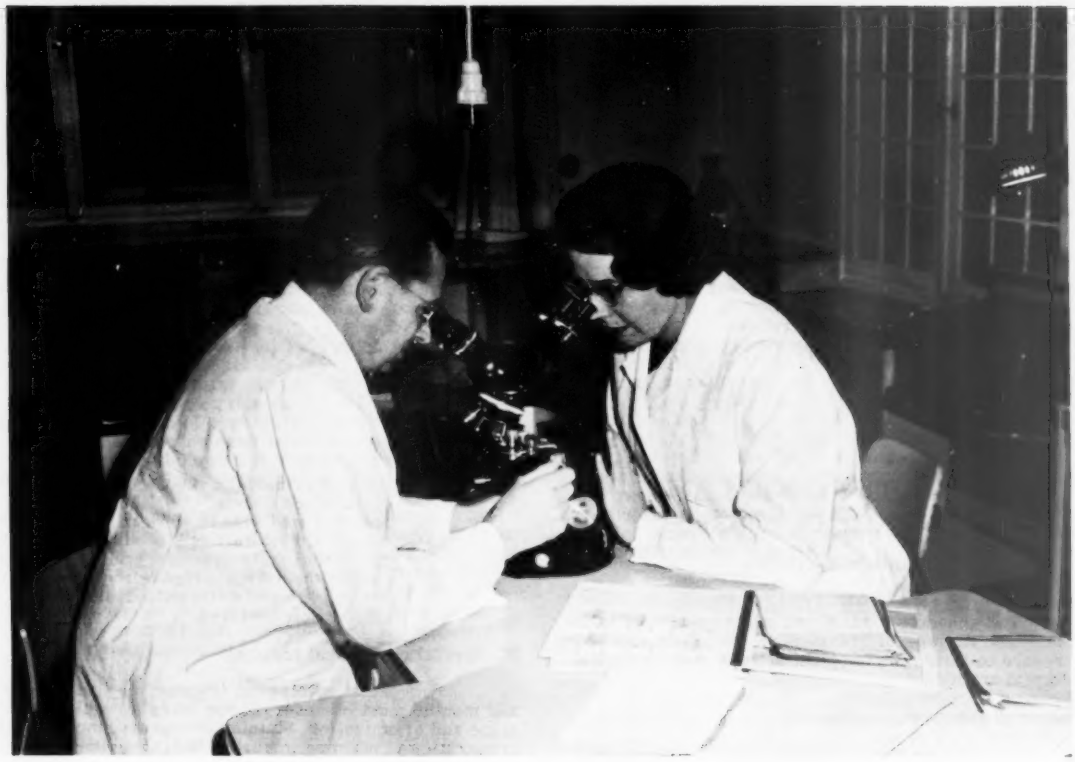


Fig. 1.

microscope, as is shown in Fig. 1, proved useful to us.

5. As far as material for simultaneous viewing by teacher and student is concerned, an orientation is also performed in other fields of cytodiagnosis (ascitic fluid, pleural fluid, breast secretions, sputa, etc.).
6. Looking at unfixed and unstained smears under the phase contrast microscope is only performed if the student is sufficiently acquainted with judging stained preparations. After that, phase-contrast microscopy is, generally speaking, not difficult.

JEAN A. de BRUX
Paris, France

In order for the cytotechnologist to acquire an intelligent understanding of his work, it is wise to teach him:

1. The essential notions of histology concerning the organs whose exfoliated cells he will be called upon to examine. These notions should always be accompanied by slides of tissue.
2. Notions of histiophysiology. It would be unthinkable to attempt to train a technician without explaining genital physiology and the hormonal actions on the receptors.

Once these notions have been acquired, the first vaginal smears will be presented to him, simultaneously with endometrial slides, in order to familiarize him with the phases of the menstrual cycle. The inflammations, parasitoses and mycoses will then be studied. This period should be of about two months' duration, and should be divided into two sub-periods as follows:

1. During the first weeks, smears carefully chosen for demonstration (such as are found in all laboratories) should be given for study, accompanied by histological slides.
2. During the second month, entire cycles should be entrusted to her, as well as routine hormonal examinations of the laboratory.

This work should of course be controlled either by the head of the laboratory or by the chief technologist.

After this preliminary period, and only if the training is excellent (this depends partly on the student, but especially on the person giving the training), one may try to push the instruction to a more advanced point, in the direction of cancer research.

This period is difficult, and necessitates the acquisition of notions on the different varieties of tumors, carcinomas and sarcomas, and benign tumors.

Pathological slides should first be explained to the technician, with relation to the architecture and cellular morphology, especially in the zones where the exfoliation takes place.

Simultaneously, he should be given smear-slides for purposes of comparison. Several weeks should be given to this training, during which the training concerning hormonal smears should also be continued and completed.

Two months at least are required, on the average, for a mastery of these larger notions. Later, finer conceptions, such as the dysplasia, the metaplasia and the pre-cancerous lesions, can be presented and defined with the aid of correlations.

Over a period of six months, the technician will have acquired an approximately complete training, but still decidedly insufficient, and he will still need another month to complete and consolidate the knowledge he has acquired. It is during this final month that an intensive theoretical and practical training in a specialized center will be most beneficial for consolidating and clearing up special points.

The amount of knowledge expected of the technician, and the duration of the training necessary to acquire it vary, of course, according to the background and intelligence of the student.


## GRACE R. DURFEE

New York, New York, U.S.A.


The academic background of students selected for training in cytotechnology should fulfill the requirements outlined by the Registry of Medical Technologists of the American Society of Clinical Pathologists. The pretechnical requirements are two years of college, sixty semester hours including twelve semester hours of biology. These requirements should be extended to include histology and chemistry plus a smattering of the related sciences.

It is felt that the pretechnical requirements of the Registry are minimal and not unduly severe and by fulfilling these requirements every cytotechnologist is eligible for the certification examination once he has acquired the stated training and experience in an acceptable laboratory. Whether or not the individual desires to take the certification examination is another matter, but at least he is prepared for it.

At the onset it is necessary to stress the importance of a quiet and congenial atmosphere in a laboratory either partially or wholly devoted to the training of cytotechnologists.

Under the guidance of a senior cytotechnologist instructor, the following program of practical training is suggested:

1. Methods of Collection: At least a day or more during the first few weeks of training is devoted to the methods and problems concerned with the collection and fixation of specimens from the various body sites. Actual observations of the collection of specimens is the most useful means of demonstration. However, if this is not possible in all cases, projection slides and drawings may be utilized effectively.
2. Laboratory Technique: An initial period of one week is spent in the preparation and staining of specimens. Laboratory procedures are explained and demonstrated step by step and throughout the training period the student should spend at least five hours a week actively participating in the technical aspects of cytology. Although mimeographed copies of the procedure may be available, they should not take the place of the student's own notebook.
3. Handling of Reports: Two or three hours of observing the methods of accession of specimens and the methods of reporting and filing of slides and reports is usually sufficient to give the student a working knowledge of the secretarial and clerical end of a cytology lab. If the training laboratory utilizes any form of punch card for obtaining data, the student should become familiar with the use and value of the method.
4. Microscopy: Students are given one-half day of instruction in the mechanics of the microscope in order to have a first hand knowledge of how to care for and get the most out of the instrument.
5. Recommended Reading: Textbooks of histology, anatomy, and cytology together with reprints are available for reading and reference.

### CYTOLOGY OF THE FEMALE GENITAL TRACT

The following outline for use in the study of the female genital tract is submitted as a basis for outlining the study of the cytology of all other sites.

1. Normal Cytology
   - History of Cytology
   - General morphology of the cell
   - Identification of common cells
   - Identification of cells and changes during normal menstrual cycle
   - The atrophic smear
   - Cellular changes during normal pregnancy
   - Cellular changes due to hormones
2. Inflammation
   - Comparison of smears in inflammation with normal smears
   - Identification of trichomonads and the cellular changes sometimes seen in the presence of this parasite
   - Effects of local treatment such as recent cautery, currettage, antibiotics etc. on the cells
   - Identification of fungus
3. Borderline Atypias of Squamous Epithelium
   - Epidermoid Carcinoma in situ
   - Invasive Epidermoid Carcinoma
4. Atypia of Endometrial Cells
   - Adenocarcinoma of Endometrium
5. Metastatic Carcinomas including ovary, etc.
   - Effects of Radiation therapy on normal and malignant cells
   - Unusual tumors of the Female Genital Tract
6. Review and an Examination based on twenty or more slides

Study sets of slides are prepared for each phase of the female genital tract and include a vaginal and cervical smear on each patient. Cards are available in each case giving the age, first day of the last menstrual period together with the history, clinical findings and description

of the cellular contents and background of each smear. Each stage of study may be prefaced by the projection of Kodachromes and a short discussion in order to orientate the students and give them an opportunity to ask questions. The instructor utilizes the daily material to supplement the study set. Interest and enthusiasm can be maintained at a high level by giving out appropriate slides for marking and identification of specific cell types. Short oral examinations daily are stimulating and worthwhile.

It will help the student's visual acuity and visual memory considerably if he writes a brief description of the various types of cells he sees in a smear or the general pattern of the smear. If he is studying a series of Trichomonas slides, he will also note that his brief descriptions contain a record of certain cellular changes common to nearly all smears containing trichomonads.

Each student's questions should be fully answered and all his difficulties clarified before he progresses to the next stage of study. The important three Cs to remember in the instruction of student cytotechnologists is the necessity of constant review, constant comparison and constant characterization of smears and cell patterns.

The time required for a student cytotechnologist to become proficient in screening cytologic material from the female genital tract varies from 4 to 6 months. In the author's experience it required six months to master the cytology of this site really well. After four months the student is usually ready to start controlled screening and by this is meant screening of the daily laboratory material which is rechecked by an instructor. The student's errors are noted and discussed with him. A student is occasionally checked by one of the professional members of the staff who plies him with questions during each of these sessions. It is during this period of practice screening that students develop judgment. They become aware of their shortcomings and recognize that any doubt concerning the negativity of a smear necessitates consultation with an experienced screener. Students at this point should have some idea of where the cytotechnologist's responsibility ends and the responsibility of the members of the professional staff begins.

At the end of the six months period of training, students should be proficient in eliminating negative smears in gynecologic material, although they still may have some difficulties with severe inflammatory atypias. Before "graduating" to actual screening each student should satisfactorily pass an examination on at least 25 slides selected for this purpose.

Using the same general outline of study as suggested for the cytology of the female genital tract, the student cytotechnologist is instructed in the cytology of all other body sites. During this period he becomes of value as a part-time screener of gynecological material under supervision and concentrates one-half of his time on study. In an amazingly short time, three months at most, he will be able to do a very creditable job of screening all types of cytologic material.

As soon as the students have fulfilled the requirements in gynecologic cytology, they are taught the cytology of other organs following the same general outline of study. Thus cytology of the respiratory, genito-urinary and gastro-intestinal tract is studied, followed by the cytology of body fluids and miscellaneous smears. During the final period of training the students are capable of offering valuable assistance as part-time screeners of gynecological material and at the same time carry on the study of the cytology of other body sites. At the end of one year each student will have become a qualified cytotechnologist.


## EMMERICH von HAAM
Columbus, Ohio, U.S.A.


Our Division of Cytology is offering a six-month approved training course in cytology, which is divided into two three-month periods, one dealing with didactic cytology and then followed by on-the-job training. The type of practical training depends somewhat upon the experience the candidate has had before enrolling in the course in cytology. In the past we accepted exceptional students with only a high school education, who had had no training in microscopic examination and had to learn the microscopic structure of cells from the ground up. This we have abandoned because of the ruling of the Board of Registry of Medical Technologists of the American Society of Clinical Pathologists, and we are now accepting only students who either are fully trained medical technologists or who have had at least two years of college work with courses in biology, bacteriology and histology. Thus we are dealing with students who are familiar with the microscope and with the microscopic structure of cells and micro-organisms.

After a short review in general cytology, we first teach the students the special staining techniques used in our laboratory. Then the students receive one month of didactic and practical training in vaginal cytology, one week of didactic and practical training in respiratory cytology, followed by one week of practical training in vaginal and respiratory cytology. The seventh week is devoted to didactic and practical training in gastrointestinal cytology followed by one week of practical training in gastrointestinal, vaginal, and respiratory cytology. The ninth week is devoted to didactic and practical training in exudates and transudates followed again by one week of practical training in the previously taught subjects. The eleventh week is devoted to practical and didactic training in genito-urinary cytology and breast cytology followed by two weeks' practical training in cytological specimens from all sources. Frequent brief examinations of unknowns are given during this course, which is concluded by an exhaustive written, practical, and oral examination. The practical training during the first three months consists of at least six hours daily laboratory work with an instructor present at all times. The specimens are taken from specially prepared teaching collections with known history and diagnosis. Ruth Graham's book and Papanicolaou's Atlas are used for reference texts.

Students who pass the first three months of combined practical and didactic training are then admitted to three months of on-the-job training. They are assigned a place beside an experienced cytotechnologist screener and re-examine the slides after the screener has finished, or the schedule is reversed. During this period weekly conferences are held in which interesting specimens are demonstrated to the group by means of the Scopicon, and the students are quizzed on theoretical subjects learned during the course. In the middle of the fifth month the students are permitted to screen vaginal smears by themselves, subject to checking by the supervising cytotechnologist.

We agree with Nieburgs (1) that training of cytotechnologists poses few problems in a laboratory where a qualified cytopathologist can direct the training, and that most difficulties arise in those laboratories where cytotechnologists are left on their own and have no persons to consult with in doubtful or difficult cases.

### Bibliography


1. Nieburgs, H. E.: Cytologic Techniques for Office and Clinic. New York, 1956, Grune & Stratton.

## SARA HODGES

### Charlotte, North Carolina, U.S.A.

After more than a decade of studying and working in exfoliative cytology, my concept of practical training has evolved from instruction of one student to a school of nine. During that time the procedures have remained practically the same.

Formal lectures on histology and cytology are given by a pathologist. The use of the microscope is also discussed and the principal mechanical features are identified. Following this, study sets of normal cells are given to the student for microscopic study. At first the student will probably find the time allocated for microscopic work unduly long, but as he becomes accustomed to the microscope and the fascination for detail that the cells inspire, he will be able to spend more time with greater ease in this phase of his work. After the study sets of normal cells have been studied and identified, malignant cells are presented and identified. We have found that having the student draw the cells in color from the microscope and labelling each type has been a satisfactory method of helping the student to notice detail of cellular form.

When the student has become familiar with the common cell types, he is given a box of known positive and negative cases. He is then instructed in the technique of screening, the process by which smear preparations are examined to detect abnormal and malignant cells. This is done by use of the low-power (10X) objective for the most part, but when more detail is needed for better identification, then the high-dry (43X) objective is used. The student is required to screen the entire mounted area by means of moving the mechanical stage from left to right and overlapping each field going from top to bottom. When abnormal cells are seen an identifying ink dot is placed to the left of the cell or cell groups for discussion later. The supervisor checks at intervals to see the progress made and to help with problems. When the study sets are satisfactorily completed, the student is given unknown smears to screen, which will be checked by a supervisor for a period of at least six months. Spot checking continues for another six months. This keeps the student and supervisor in close contact and encourages stimulating discussions.

During the training period the student participates in all phases of the cytology laboratory. This includes staining technique, preparation of smears from all body fluids, filing and cross-filing systems.

## PATRICIA KELLY

### San Francisco, California, U.S.A.

The practical training of cytotechnologists is a two-way venture in which the instructor presents the material to the students and the student integrates the information into good working habits at the microscope. The program, therefore, consists of 1) a well-planned method by which the material is presented to the student and 2) an effort on the part of the student to ever-increasing awareness of minute detail in the examination of cells.

I. The presentation of material

In my experience, the teaching program has always been a blend of three things: 1) lectures and theoretical instruction by cytologists, 2) personal supervision and training in technical laboratory procedures by cytotechnologists and 3) the examination of cells by the student.

Each major section of the work is introduced by a lecture and additional lectures are given as the study progresses. During this period the student examines assigned teaching slides and a cytotechnologist is always available to give the personal instruction such study requires.

Weekly examinations and seminar discussions enable the instructor and the student to pinpoint difficulties. This is more easily accomplished when the student describes, not only the type of cell in question, but also the cellular features which lead to her conclusions.

Visual Aids

Photomicrographs. Repetition is a necessary part of instruction and photomicrographs provide a means of diversified repetition as well as good illustrations for lectures. I prefer Ectochrome transparencies at three different magnifications: X400, X600 and X900. In photomicrography this gives the impression of low power, high power and oil immersion, and permits the proper orientation of the cell before examining cellular detail under higher magnification.

Charts. Through the use of charts the distinguishing features of cells may be visualized in minute detail. At the same time, it unifies salient ideas regarding the cytologic, histologic and pathologic position of the cell in its site of origin. In addition, they serve as a permanent reference center to which the student may return whenever the need arises.

Reference Library. A comprehensive reference library is an important teaching adjunct. Many excellent books on exfoliative cytology are now available and should be within easy reach of students in every teaching laboratory. An up-to-date reprint file is equally important. Reprints enable the student to keep abreast of the latest advances in the field of cytology, acquaint her with the work being done in other laboratories and encourage the idea that future study is necessary after completion of the four month course.

II. The screening process

When the student has satisfactorily completed the examination of study slides, she is ready to screen under supervision. For the purpose of clarity it is well to view this work from two different aspects: 1) the examination of individual cells and 2) the examination of smear patterns. In practice, however, the two parts overlap.

The examination of cells requires specific observation of 1) the nucleus, 2) the cytoplasm, 3) the nucleus in relation to the cytoplasm, 4) the cell in relation to proximate cells, especially in aggregates, and 5) the cell in relation to the smear as a whole.

In screening, some cells encountered may not be well-differentiated. These cells should be dotted by the student and compared with other cells, on the same smear, whose distinguishing features are intact. By comparing undifferentiated with differentiated cells, the student will gain a greater understanding of the range of normal cells and of cells showing benign atypias as well as malignant changes.

The cytotechnologist has the advantage of examining the whole smear, and for this reason the student will become familiar with smear patterns. This is especially true in cases of microbiological and hormonal evaluation and in cases of pregnancy and abortion, where the entire smear, as well as individual cells, must be considered.

Although screening will provide the maximum benefit in understanding cells, in my opinion it is misleading to set a time limit and say, "A student cytotechnologist will learn cytology in four months—or six months." If this were true, exfoliative cytology would be a subject with very limited boundaries. This, of course,

is not the case. The cytotechnologist too must always remain a student, alert to the new and the unusual. This search for the unknown should be insatiable—the fuel that keeps enthusiasm in high gear.

PASCO R. del VECCHIO
Bethesda, Maryland, U.S.A.

The practical training program of cytotechnologists should consist of lectures and textbook and laboratory studies with demonstrations and examination of slides. The training should cover a period of six months for vaginal and cervical cytology, to one year for vaginal, cervical and extra-uterine cytology.

The lectures should include a history of cytology, its aims and purpose, the proper use of cytologic terminology, explanation of methods used in the preparation of specimens and a brief review of the anatomy and histology of the site from which cytologic material is obtained. The lectures should be correlated with the type of cytology being studied and students should be given textbook and journal assignments accordingly. Periodic examinations both written and oral should be given to evaluate the progress of the student.

The laboratory study should begin with the proper use and care of the microscope. All laboratory work should include step by step demonstrations by the instructor of all procedures. The importance of each step in the procedure should be explained in terms of its relationship to the final product to be examined. The laboratory workshop should cover the following: 1) the preparation of solutions; 2) the proper care and use of laboratory glassware and equipment; 3) the collection of specimens both vaginal and extra vaginal, and 4) the methods for processing specimens, including: a) centrifuging, b) use of the Millipore filter, c) cell blocking, d) preparation of smears, e) fixation of specimens and the proper fixative to use, f) knowledge of various stains and staining procedures, g) the proper method for labeling and coverslipping specimens, and h) the importance of proper recording and record keeping.

The microscopic study of cytologic material should begin with a demonstration of the proper screening technique. The student should first be taught the cytology of the normal vaginal tract. Emphasis should be placed on the effects of hormonal changes, bacteria, fungi and parasites on the over-all cytologic picture and the changes noted in the individual cells. An instructor should be available to assist the trainee with the interpretation of the material being studied. The study and identification of atypical and neoplastic cells should begin when the student has proven ability in identifying normal cells. The instructor at this time should try to give individual explanations to each student as to the criteria of malignancy through cytologic demonstrations. During this phase of training the student should be allowed to study as many identified neoplastic cells and as much positive material as possible. This enables the trainee to become familiar with many of the different types of neoplastic cells. Here again the use of periodic tests are of great value in keeping the instructor informed as to the progress of the trainee and familiarity with the morphologic characteristics of the cells. The trainee should now be ready to identify and evaluate with a written report sets of training slides. In interpreting these slides as negative, suspicious or positive, the student must give a full description of the specimen and be able to identify the findings described in the report. The evaluation and presentation of a written report on the daily work submitted to the laboratory should begin when the trainee has demonstrated cytologic proficiency with the training sets. For a period of three to six weeks the written reports and specimens should be checked by the instructor. Any differences in the evaluation should be reviewed and discussed with the trainee. The student should now be ready to begin assuming the initial responsibility of evaluating cytologic material. After completion of a training program of this sort the student should be well qualified as a cytotechnologist.

## DISCUSSION

**MARGARET E. ATTWOOD, Birmingham, England, U.K.:**

It amazes me to find how much I disagree with the main speakers on one particular aspect of their training program.

In England there is not, and probably never will be, such a person as a cytotechnologist, if by this we mean a specialist trained in only this one subject. We have been training students now for eight years, some of them clinicians, some pathologists and many technicians. From our experience we are quite convinced that cytological examination of smears can safely be left in the hands of trained technicians. In addition to cytology, our own technicians are taught the discipline of routine laboratory work in pathology, bacteriology, biochemistry and hematology. After three years of general training they may specialize in one of the above subjects, with the exception of cytology. Cytology is not yet recognized as an examination subject in the curriculum of the Institute of Medical Laboratory Technology, whose membership our trainees are expected to attain.

In our Department of Obstetrics and Gynecology cytological specimens are collected from the clinics by technicians who screen and report on the slides, at the same time keeping records for routine and research purposes. Any tissue, e.g., from a ring biopsy, is processed for histological examination by the same technicians, which, I think, greatly adds to the interest of their work.

I feel that all the speakers have laid too much stress on the theoretical aspect of training in cytology and not sufficient emphasis on the technique of identification and recognition of cells under the microscope. This is a technique (as with other technical subjects) at which some people are naturally gifted and will become safe and accurate while others, no matter how comprehensive their background training, will have no aptitude. It is essential, therefore, that great care be taken in the initial choice of a trainee. I have found that a good student laboratory technician is most likely to make a good trainee in cytology.

I would agree with Patricia Kelly that the greatest factor in training is constant personal supervision by a cytologist in the early stages, with daily tutorials around the microscope, and later with discussions on particular slides. I agree with many of the speakers that initial training should be on cells from the female genital tract. One is primarily concerned in training the eye of the cytology technician to recognize the import of the most minute change. Once this basic technique is acquired a student may proceed with confidence in other fields.

Von Haam mentions the difficulty of cytology technicians working in isolated hospitals. Our experience in the Midlands has shown that close liaison should exist between such workers and an experienced cytologist, although of course in their own hospitals they work as a team with the clinician and the pathologist.

**J. ERNEST AYRE, Miami, Florida, U. S. A.:**

Premedical training or previous training in pathology provides good background for more specialized technical training in cytology. Grace Durfee has outlined in a comprehensive manner the scope of practical training for screeners. The latter program seems to be more adequate.

IRENA KOPROWSKA, Philadelphia, Pennsylvania, U. S. A.:

I should like to comment on specific points of programs for the practical training of cytotechnologists, as outlined by some of their proponents:

1. An excellent didactic method, which Bickenbach and Soost use, viewing smears with students through a special double microscope, may put an excessive demand on the busy schedule of most cytopathologists in training centers in this country. As an alternative, we found it profitable to provide students with smears which have cells marked on the slides. These cells are described on corresponding cards and accompanied by self-service Kodachrome sets containing enlarged pictures of important cells in a given slide. Much time and effort are undoubtedly required for the preparation of such sets, but subsequent instruction is less time-consuming to the instructor and very gratifying to students. Students who advance to the stage of actually pre-screening routine laboratory cases, mark cells with a different color ink than that used by qualified screeners who subsequently see these smears. After the final cytologic report is issued, students compare their marks with marks of screeners and with the final report of the cytopathologist. Cases needing further clarification are discussed accordingly.
2. I am in full agreement with Grace Durfee's suggestion to provide ample opportunity for screening gynecologic specimens before theoretical instruction is given in other types of secretions. Until 1959, we followed another system, presently advocated by von Haam. This consisted of alternating didactic and practical teaching of all types of cytologic material presented in quick succession during a three-month period, and followed by three months of straight screening. Last fall, however, we started experimenting with the system presently advocated by Grace Durfee without previous knowledge that it had been used at her laboratory. The results have been most gratifying.

Our own variety of practical instruction is integrated with a theoretical teaching program in its present form and includes a reversion of the conventional order of training. Customarily, theoretical instruction precedes practical exercises with emphasis on introduction to the subjects through lecturing. Since the fall of 1959, students of Hahnemann Medical College School of Cytotechnology, following a single introductory discussion with the instructing cytopathologist, were exposed to the initial hardship of plowing through assigned literature and the collection of slides and Kodachromes by themselves. They were then left at the discretionary guidance of experienced cytotechnologists and cytopathologists in training. At certain predetermined turning points of this guided self-instruction, students are subjected to brief, individual oral and practical examinations. During the practical examinations, students are asked to identify cells in colored transparencies (Kodachromes) shown by the instructor. Only after the desired quality of knowledge, painfully acquired by the student, becomes apparent to the examiner does the latter undertake individual teaching for the benefit of each student. The purpose of this is to fill any gaps discovered in his knowledge. A rapport, which develops between the cytopathologist and each student, helps to develop and maintain students' interest in and understanding of important problems in exfoliative cytology. Students' horizons are noticeably broadened during such sessions, as their eyes open to the existence of related scientific pursuits, and to the epidemiology of diseases under investigation.

Although this system of combined practical and theoretical training needs further testing, it seems that the desire and ability of students to assimilate subsequently-given instruction are stimulated by their initial struggle to pick up needed information the hard way. Since individual instruction is provided in preference to group teaching, such a program is admittedly of limited applicability. It would be especially impractical in teaching centers accepting a sizable number of students.

C. PASCAL, N. DENAVIT and L. TRONCY, Lyon, Rhône, France:

The problem of the practical training of cytotechnologists has been extensively considered by the above authors. This training is certainly much more important than the theoretical teaching. We agree that it will be useful to be familiar with the clinical work: The cytotechnologist must at least have a good knowledge of the different procedures used in obtaining the specimens, and he must know the terminology of colposcopy. However, we do not believe that the cytotechnologist should take the specimens himself. He certainly has enough to do with his work in the laboratory.

A six-month training period appears to us to be a minimum in order to obtain some practical experience. However, this will depend on the individual student. In our opinion, the practical training must begin with the cytohormonal evaluation: the cytotechnologist should perform cell counts until the figures obtained are similar with those already established by a well-trained cytotechnologist. Detection of cancer cells will require more experience and six months will not be enough time to develop an efficient screener. We suggest beginning the training by showing an extensive series of cytologic microphotographs. However, diagnosis cannot be learned from microphotographs alone. Drawing of cells and especially nuclear structure by the student himself under the oil immersion will be helpful. Then the regular screening work can be started. We suggest that the slides be carefully reviewed before by the cytologist, who identifies all interesting cell elements with the aid of the platinum micrometer, and retains this identification for himself. The cytotechnologist must then find these areas and mark them. It is then easy to evaluate by comparison whether or not the screening has been performed satisfactorily.

Finally, the cytotechnologist may be asked to try to classify the slides. This is not necessary however, since cytotechnologists should not give any final diagnosis.

We believe that even after an extensive training time the cytologist should continue to control for some time the slides screened by the cytotechnologist.

HOWARD L. RICHARDSON, Seattle, Washington, U. S. A.:

The philosophy of training as expressed by the seven authors is similar. However, the adoption of the requirements as outlined by the Registry of Medical Technologists of the American Society of Clinical Pathologists has materially aided in the standardization of thought and discipline in the United States of America. Grace Durfee has amply brought out one of the problems that face pathologists: "Students at this point should have some idea of where the cytotechnologist's responsibility ends and the responsibility of the members of the professional staff begins." Too often the cytotechnologist, after a period of training, becomes a diagnostician and the results of cytologic screening are made known to physicians who may not have the training or the responsibility in pathological diagnoses. Because of the complex interpretations in the twilight zone of atypia, this has caused the

method, in some communities, to fall into disrepute and has hindered progress in widespread adoption for the early diagnosis of cancer.

It is noted with extreme interest that no one has mentioned the psychological evaluation of the individual who is to become a successful cytotechnologist. In my experience, the hard conscientious worker who has a picture memory pattern and profits by mistakes will be a cytotechnologist of merit.

EDMUND SCHÜLLER, Vienna, Austria:

One point belonging to the practical training seems very important: to eliminate pupils who are absolutely unfit for microscopic work. With no visual memory or visual talent one cannot become a cytotechnologist. Even very intelligent people often lack such ability, so it is not, in any sense, a value judgment of personality. One can only make such a selection if one has personal contact with every pupil from the beginning of practical training. Only in this way can one prevent unfit people from obtaining a degree as cytotechnologist, or prevent the useless expenditure of vast amounts of time on long training periods.

JOSÉ R. DEL SOL, Madrid, Spain:

We consider that a minimum time of one year divided into three periods is necessary for the proper training in cytotechnology:

First period. This section of the practical program is dedicated to familiarization of the future cytotechnologist with the laboratory organization, preparation of staining procedures, elementary optical study, filing and classification of material, initiation in microscopic observation, personal contact with the Out-patient Department, preparing cytology specimens, etc.

Second period. The junior screener writes down the findings which will be corrected by a senior screener. During this period, personal contact with the laboratory chief is established.

Third period. The last period is dedicated to working as a senior screener under supervision by the laboratory chief, and she is responsible for the quality of staining.

MARVIN W. STAFFORD, Philadelphia, Pennsylvania, U. S. A.:

I wholeheartedly second Grace Durfee's comment concerning the importance of a quiet, congenial atmosphere in a laboratory devoted to the teaching of cytotechnologists. One of the more important things that the neophyte cytotechnologist must develop is the ability to concentrate on the case at hand. In few other technical fields does responsibility weigh as heavily on the shoulders of the individual. Therefore, conditions under which the cytotechnologist is trained become extremely important.

Even though the prime mission of cytotechnologist training schools is to provide workers for the purpose of detecting cancer, the area of cancer research cannot be ignored. This idea is touched upon by de Brux in his paper. In our training course, the student cytotechnologist, as a requirement for graduation, must complete a minor research problem. Thus exposed to research procedures, the cytotechnologist becomes a more versatile addition to the staff of the pathologist.

As illustrated by the above authors, there is no cut and dried time schedule that will automatically produce the polished, accomplished cytotechnologist. The development of the all-important (sometimes elusive), self-confidence in the student depends on many variables, the most important of which is the ability of the instructor.

Any basic training course should be considered just that, basic training. The training and development of the good cytotechnologist never stops. Every laboratory has the responsibility of providing means of continuing the education of its technical staff in order to keep abreast of this ever-changing, ever-progressing field of cytology.

CHARLOTTE M. STREET, New York, New York, U. S. A.:

All programs suggested are quite similar in the essential points and very good in most respects.

The extensive premedical education advocated by Bickenbach and Soost doubtless provides a good background for cytotechnology, but might well be replaced by lectures and demonstrations. The length of time assigned to learning routine and organization seems unnecessarily protracted. Microscopic interpretation is the most important and most difficult part of the training and should receive the greatest emphasis.

The plan outlined by de Brux is very comprehensive but applies only to gynecological cytology. It could, however, be followed for other applications.

Von Haam's program is very thorough and intensive, but, judging by our experience, only one week for didactic and practical training in each of the four non-genital applications seems rather short if the student is expected to study a mixture of slides each second week. One week might be added to each of these periods, leaving two months for on-the-job-training.

Grace Durfee, Sara Hodges, Patricia Kelly and del Vecchio have suggested excellent programs.

Not all speakers mentioned practical examinations, but I consider them indispensable for an evaluation of the student's progress and his need for further instruction or clarification of problems.

DOROTHY B. WELLER, Providence, Rhode Island, U. S. A.:

Grace Durfee has offered a complete and concise outline for a practical course in cytology. I agree with her recommendations and would emphasize her suggestion that the student's questions and difficulties be clarified before progressing to a further stage.

It is important, as von Haam mentioned, to have an experienced instructor constantly available to the student. Weekly conferences are valuable and necessary, however. Since many uncertainties occur to the student while she is studying at the microscope, that should be the time for immediate clarification.

As opposed to a schedule presenting successive weekly periods devoted to the study of specimens from various sources, I believe it is less confusing to the beginner if he first becomes proficient in the cytology of the female genital tract, and then instruction in extravaginal cytology proceeds easily and rapidly.

In my experience, many of the fundamentals of cytology are taught verbally, and although this early instruction is the easiest for the student to understand, its presentation takes proportionately more of the instructor's time. I often think that a new textbook would be helpful, introducing practical cytology at a basic level, to be studied as a preliminary to the excellent texts now available, which are suitable for study when the student is a little more advanced.

## CLOSING REMARKS

WERNER BICKENBACH and HANS-JÜRGEN SOOST:

The discussants felt that the microscopical training and experience in the evaluation of the cytological specimens should find the greatest emphasis in the practical training program. We also consider this to be correct. The practical microscopical training should

take at least half the time of the complete training program.

PATRICIA KELLY:

Although there seems to be some divergence in individual application, it is interesting to see that there is general agreement on the essential points of the practical training programs.

Pascal, Denavit and Troncy have added many excellent recommendations. The drawing of cells by the student is a particularly profitable exercise; one which I have found of great benefit.

Also, the combination of histology and cytology as suggested by Margaret Attwood could lend great interest to the work, but this practice may not be feasible in all laboratories. However, I do not find it possible or desirable to draw such a sharp division between theoretical and practical training.

Charlotte Street has stressed the importance of practical examinations. I agree with her completely. In addition to clarifying problems, they give added incentive to the student who is making good progress.

Schüller and Margaret Attwood put first things first when they emphasized that care must be taken in the selection of trainees. Unless the student has a definite aptitude for cytology, efforts in practical training will be fruitless.

GRACE DURFEE:

See following topic.

# PROGRAM OF THEORETICAL EDUCATION FOR CYTOTECHNOLOGISTS

WERNER BICKENBACH AND
HANS-JÜRGEN SOOST

Munich, Germany

The theoretical education should be divided into two sections: (A) the general medical pre-education and (B) the special training.

## A. GENERAL MEDICAL PRE-EDUCATION

1. General teaching about obligations and duties of medical aides, e.g., information about physicians' discretion, communication with patients, introduction to work which is directly connected to patients, medical terminology, etc.
2. Basic anatomy and physiology of the human body.

The following details are for students who wish to work in the area of gynecological cytodiagnosis. For other areas of cytodiagnosis the teaching schedule would have to be changed.

3. Anatomy and physiology of the female genital tract, including histology (extensive).
4. Important gynecological diseases and their treatment (e.g., benign and malignant tumors of the female genital tract, physiology and pathology of vagina, the so-called erosion of ectocervix, pregnancy and its disturbances).
5. Selected topics of endocrinology (physiology of the menstrual cycle, hormonal conditions in the various periods of life, important hormonal disturbances of women).
6. Some basic conceptions of histology (what is a carcinoma, a sarcoma, what is an atypical epithelium? etc.).
7. Basic conceptions of colposcopy (the cytotechnologist should not learn to interpret colposcopic findings; but he should know what colposcopic diagnosis means, e.g., ectopy, epidermization).
8. Care of the microscope.

## B. SPECIAL CYTOLOGICAL TRAINING

The theoretical teaching of gynecological cytodiagnosis should include:

1. Taking, fixing and technique of mailing cytological preparations. Staining after Papanicolaou in addition to some other staining methods. General facts for evaluating cytological preparations.
2. Morphology of the normal cells of vaginal epithelium, the endocervix and endometrium also something about blood morphology.
3. The vaginal cell patterns in the various ages of female life.
4. Changes of vaginal cell pictures during the artificial application of hormones.
5. Non-hormonal factors which influence the cell picture; bacterial cytolysis, degenerative autolysis, bacterial and other infections (Trichomonas, etc.), additional inflammatory changes, etc.
6. Changes of the cell pictures due to artificial influences and technical mistakes.
7. The vaginal cell pattern in hormonal disturbances.
8. Sex chromatin examinations.
9. The vaginal cell pattern during normal pregnancy, and its changes during puerperium and abortion.
10. Changes of the cell pattern with the presence of inflammatory and other gynecological diseases.
11. The cytological diagnosis of carcinoma with critical discussion of malignancy of the single cell, of the atypical cell patterns in general, the interpretation of doubtful smears, the importance of cytological findings for the clinician and a review of the abilities of cytodiagnosis in searching for traces of cancer.
12. Cell changes after radium and x-ray treatment.
13. Cytology other than gynecological (ascites, lung, stomach, urinary tract, breast secretions, etc.).
14. Usage of phase contrast microscopy.
15. Organization and working methods of a cytological laboratory.

## MARIA S. BLANCO de del CAMPO

### Palo Alto, California, U.S.A.

The program I shall outline is designed for the student who will undergo a traineeship of six months under the supervision of a pathologist—after completion of six months of theoretical education in an approved school for training of cytology.

The course, divided into two parts, presents in the first half facts and principles of exfoliative cytology, procedures for collection and processing of samples, and mechanisms in screening; the second half provides opportunity for independent thinking and responsible screening. I suggest lectures in the following topics and in this sequence: 1. Introduction to Exfoliative Cytology; its goals, its history and the significance of the method. 2. Introduction to techniques in microscopy and the processing of smears. (Evaluation of different methods for staining, collection of specimens, chemistry of stains, etc.) 3. The cell. 4. The epithelium. 5. Histology and anatomy of the female genital tract. 6. Normal epithelial and non-epithelial elements of cervical, vaginal and endometrial smears. 7. Infectious organisms and host-response depicted in smears. 8. Patterns in hormone evaluations from childhood to senility, including pregnancy, responses to hormone therapy, and deviations from normal. 9. Patterns in cancer. Include biological characteristics of cancer cells and problems in cancer research. Criteria for recognizing and distinguishing various types of tumors such as squamous cell carcinoma and adenocarcinoma. 10. Patterns in dysplasia. 11. Radiation response. 12. Pulmonary cytology. 13. Gastro-intestinal tract cytology. 14. Breast and prostatic cytology. 15. Cytology of the urinary tract. 16. Cytology of effusions. 17. Peripheral blood cytology. 18. Determination of sex chromatin.

Lectures are supplemented by movies, Kodachrome slides and laboratory exercises. Written examinations are given at the end of each topic. Students are encouraged to participate by presenting seminar papers and discussing slides-of-the-week in round table discussions.

## GRACE R. DURFEE

### New York, New York, U.S.A.

The theoretical education in the training of cytotechnologists parallels the practical training. A general knowledge of histology and anatomy form the basis for the study of cytology. Assigned reading in histology and anatomy textbooks combined with formal lectures and informal discussions and the use of drawings, pictures or projection slides help the student to grasp the essentials. In order to relate the three subjects as a whole, anatomy and histology of specific organs should be taught concurrently with the cytology of that site.

Periodically, members of the professional staff may offer informal lectures on topics of particular interest with the aid of Kodachromes. Such topics are as follows:

1. The cytologic and pathologic findings in a particular tumor.
2. Preview of a paper which will be given at a meeting.
3. Current research projects.
4. Projection and explanation of a set of kodachromes to be used for a seminar.

These sessions are followed by a question and answer period.

Discussions and reading assignments in general endocrinology are of value particularly as related to the cytology of the female genital tract.

If at all possible, the members of the professional staff should give the student an idea of the basic concepts of the structure and behavior of tumors in general.

## EDMUND SCHÜLLER AND MARGARETE PEUTSCH

### Vienna, Austria

Theoretical training should begin with the principles of the normal anatomy and histology of the female genital organs, and the pathological anatomy and histology as far as is concerned with the cytology. Then the trainee receives formal descriptions of the normal common cell types. After a theoretical course in hormonal cytology the theoretical training in cancer cytology follows. Of course, the theoretical education is connected with daily practical training.

## DISCUSSION

ALICE D. CLARK, Chicago, Illinois, U. S. A.:

Everyone except Maria del Campo seems to feel that the theoretical course in the education of the cytotechnologist should be paralleled by some sort of practical experience. From Bickenbach and Soost's presentation, I am not sure of their feelings on the subject.

Schüller and Margarete Peutsch provide a program in gynecological cytology only. Do your cytotechnologists have little or no training in the other areas of cytology during their formal educational period? How long does your training period extend?

Bickenbach and Soost give the most extensive list of subjects for consideration; however, their program includes general medical pre-education. Is this general medical pre-education a prerequisite or is it an integral part of the theoretical education of the cytotechnologist as such? If it is a part of the theoretical program, how much time do you allow for the entire education? Only Topic 13 of Special Cytological Training is concerned with other than gynecological cytology. Does this mean that separate training programs should be given depending upon the area of cytodiagnosis in which one is specializing?

Maria del Campo's and Grace Durfee's programs seem to turn out the most well-rounded cytotechnologists if followed closely. My only question in the former's program would concern separation of the theoretical and practical programs and the end result, and in Grace Durfee's, the matter of time involved, of which no mention is made, and of a more specific schedule to be followed in the educational program.

EMMERICH von HAAM, Columbus, Ohio, U. S. A.:

Our training course aims to train cytotechnologists in all phases of cytology, not only in gynecological cytology as Bickenbach and Soost outline. Our students receive their theoretical education parallel to the practical training as outlined by Grace Durfee. They are given lectures on anatomy, physiology and pathology of the genital tract, respiratory tract, gastrointestinal tract and urinary tract at the time they are studying the normal and abnormal cytology of the respective systems. One month is devoted to lectures on the female genital tract and two months are devoted to theoretical instruction of the other

organ systems considered important to a cytotechnologist. In addition, they receive instruction in basic cytology and basic oncology.

IRENA KOPROWSKA, Philadelphia, Pennsylvania, U. S. A.:

Bickenbach and Soost include under their general medical pre-education, i.e., the first part of their program of theoretical education for cytotechnologists, such courses as anatomy, physiology, histology and pathology, which other authors mentioned among educational prerequisites for cytotechnologists in another section of this Symposium. This makes one realize that there is a general agreement about the need for cytotechnologists to know the anatomy, histology, physiology and pathology of the organs from which they study exfoliated cells. There is a difference of opinion as to who should teach these subjects and when and where they should be taught. The forementioned authors accept high school graduate students (see their discussion of prerequisites: "Mittelschule" is an equivalent of high school) whom one should, in their own words, "avoid burdening with too many teaching subjects" up until then. Subsequently, they expose such an individual, carefully protected from the trauma of training in medical technology, to their own program of medical pre-education.

It would be impractical for a School of Cytotechnology in this country to assume the responsibility for laying the groundwork of necessary theoretical fundamentals. These would include the teaching of anatomy, physiology, histopathology and endocrinology, as well as colposcopy, principles and techniques of exfoliative cytology, use of phase contrast microscopy and the organization of a cytology laboratory, to high school graduates without former adequate educational background and laboratory experience. Several instructors well versed in basic sciences would be required to provide such an adequate theoretical course, and many hours of formal lecturing would be needed to adequately bridge the gap between a high school graduate and an enlightened cytotechnologist. I ponder how much time such a theoretical course, combined with practical screening instruction, would involve. How comprehensive is the view of the field of Exfoliative Cytology in the mind of a high school cytotechnologist who completed, perhaps, a too-accelerated course of proposed theoretical education?

While the above authors outlined a theoretical program for teaching gynecologic cytotechnology only, Maria del Campo also includes in her program teaching of non-gynecologic cytology. This, of necessity, provides a somewhat less-detailed orientation toward clinical gynecology, with which, presumably, most currently-approved training centers in this country are reconciled. My main reservation about the lecturing program is the disproportionately small degree of attention focused on respiratory cytology in consideration of its increasing importance. Also, if students had anatomy and histology included among prerequisites for admission, as advocated by Grace Durfee, there would be no need to devote two of the total of 16 hours lecturing time to the instruction of these subjects. The necessary refreshing and supplementing of former rudimentary knowledge in these fields can be achieved by encouraging students to read assigned chapters in standard basic science textbooks. More lecturing time could be profitably devoted to Exfoliative Cytology proper with greater emphasis on pulmonary cytology.

In reference to Grace Durfee's program, we are happy to see that assigned reading of selected chapters in anatomy and histology is combined with formal lectures and informal discussions of cytology of pertinent organ systems. There is a distinct stipulation that theoretical teaching is integrated with practical training and that it is a joint undertaking of cytotechnologists and medical staff. We consider the proposed program very beneficial and, in many respects, similar to our own manner of teaching. More details concerning theoretical instruction as integrated with practical training at The Hahnemann School of Cytotechnology are given in the preceding discussion.

HERBERT E. NIEBURGS, New York, New York, U. S. A.:

The above presentations on theoretical education and practical training for cytotechnologists are well outlined. Much of the curriculum included for theoretical and practical training may be beyond the comprehension of some cytotechnologists, and it is my opinion that a training program should differ in accordance with the background and ability of the technician in training. Since there is a place for screening technicians who simply select specimens with abnormal cells from routine smears, the training should be directed strictly towards the accomplishment of this task in order to avoid any unnecessary confusion for the individual in training. Before training, the potential of the cytotechnologist is not always clearly evident, and is not necessarily dependent upon previous training or on superior intelligence. It has been my experience that the potential microscopic ability in cell interpretation may be evaluated to a certain extent by testing the technician's skill at drawing a cell or a group of cells while visualizing them through a microscope. The quality of reproduction of cellular details is usually a fair indication as to the category for which a technician may be best suited.

The duration of training in relation to the curriculum suggested in the above presentations may be too short with the use of visual memory training methods even for cytotechnologists with the highest qualifications. Visual memory necessitates extensive attention to the examination of specimens from different sites. Even an outstandingly well-qualified cytotechnologist would have a long and tedious road ahead with this approach.

The training period can be markedly reduced by teaching cytology according to definitive criteria (see Table I). Practically, this is carried out by the teaching of cell morphology at the onset of training. Simple cell analysis of specimens from all sites is practiced until the technician has learned to identify and evaluate the components of cell structures. This type of training eliminates the well-known specialization of cytotechnologists in any particular field of cytology. The comprehension of cell structure by this method may usually be accomplished in one to two months or frequently in a shorter period. The subsequent training may be directed to specific aspects of cytology and is usually accomplished within a relatively short period. This type of training usually produces a cytotechnologist with a superior knowledge of cytology.

C. PASCAL, N. DENAVIT and L. TRONCY, Lyon, Rhône, France:

Bickenbach and Soost in our opinion give an excellent and complete description of the make-up of the theoretical training which should be given to cytotechnologists. It seems difficult to add any more to their program. However, we believe that it will take more than six months to achieve their goal. With Grace Durfee we suggest that the anatomy and the histology could be taught simultaneously with the cytology of that particular organ. We believe that these studies are not necessary (but useful) for the screener. Nevertheless, his interest in his work will certainly increase with the theoretical knowledge he may gain. Before establishing a theoretical program, the role of the cytotechnologist should be clearly defined. We hope that this definition will be given in this Symposium.

MARVIN W. STAFFORD, Philadelphia, Pennsylvania, U. S. A.:

The general medical pre-education as outlined by Bickenbach and Soost emphasizes a very important point that is all too often overlooked in the training of the

Table I

**I. CELL TYPES**

Squamous

1. Superf.
2. Intermed.
3. Parabasal
4. Columnar
5. Cuboidal
6. Undiff.

**II. NUCLEUS**

A. Size

7. Normal
8. Incr. sl.
9. Incr. mod.
10. Incr. mark.
11. Nucl. cytop. ratio-normal
12. Nucl. cytop. ratio-abnormal

B. Membrane

13. Perinucl. halo
14. Nucl. membr. indentations
15. Indistinct
16. Dist.-not incr.
17. Dist.-incr. sl.
18. Dist.-incr. mod.
19. Dist.-incr. mark.
20. Border fragm.-tot.
21. Border fragm.-part.
22. Diam. var.-pres.
23. Diam. var.-abs.
24. Discontinuous

C. Nuclear Material Homogenous

25. Normal
26. Decr.
27. Incr. sl.
28. Incr. mod.
29. Incr. mark.
30. With focal areas of nucl. densities

Heterogenous

31. Ptcl. uniform-not incr.
32. Ptcl. uniform-incr.

Anisochromatosis

33. Ptcl. incr. size & var. sl.
34. Ptcl. incr. size & var. mod.
35. Ptcl. incr. size & var. mark.
36. Karyosome (Ans. also 49-53)

Total Amt. of Nucl. Mat. (Pert. 31-36)

37. Normal
38. Incr. sl.
39. Incr. mod.
40. Incr. mark.
41. Decreased

Chromatin Bands (Not pertaining to nucleolus or doubtful karyos.)

42. Interptcl. bands abs.
43. Interptcl. bands present
44. Interptcl. bands with ext. to border
45. Areas between ptcls. & bands clear
46. Areas between ptcls. & bands not clear

**III. NUCLEOLUS**

A. Size and Number

47. Single
48. Multiple
49. Normal size
50. Decr. size
51. Incr. sl.
52. Incr. mod.
53. Incr. mark.
54. Transluc. incls.
55. Eosinophil. incls.
56. Regular incls.
57. Irregular incls.
58. Anisonucleolosis

B. Nucleol. Assoc. Chromatin

59. Absent
60. Pres.-Distr.-normal Size-normal
61. Size-incr. sl.
62. Size-incr. mod.
63. Size-incr. mark.
64. Pres.-Numb. Ptcls. Decr. Size-normal
65. Size-incr. sl.
66. Size-incr. mod.
67. Size-incr. mark.
68. Round, deeply stain. or F. pos. (Nucleoli not vis.) Synon. $\bar{c}$ 36

C. Connecting Bands

69. Absent
70. Pres., reg. to ptcl. &/or c ext. to membr. $\bar{c}$ halo around nucleol.
71. Pres., reg. to membr. $\bar{c}$ or $\bar{s}$ halo but with clear spaces
72. Pres., irreg. to ptcl. &/or $\bar{c}$ ext. to membr. $\bar{s}$ halo.
73. Conn. bands distinct-thin
74. Conn. bands distinct-incr. diam.
75. Conn. bands indistinct-incr. diam.

**IV. VACUOLUS**

76. Nuclear
77. Small
78. Large
79. Cytoplasmic
80. Small
81. Large
82. Size var. abs.
83. Size var. pres.

**V. NO. OF CELL NUCLEI**

84. Single
85. Multinucleation $\bar{s}$ anis.
86. Multinucleation $\bar{c}$ anis.

**VI. ANISOKARYOSIS**

87. Abs. (If var. in size & num. below 25%)
88. Pres. (If var. in size & num. above 25%)

This table is referred to in the discussion of Dr. Nieburgs.

cytotechnologist. This is the concept of studying the cancer patient as a "whole," not just some isolated anatomic area. Anatomy, physiology and most especially, histology are indispensable requisites for the thorough education of the cytotechnologist. To further this "broad background" concept, we also include lectures in epidemiology of the various cancers, and even discuss the psychological implications that cancer may produce in the patient.

A useful study technique that we have employed with excellent results is the abstract file. There are numerous reprints assigned as supplementary reading. The student writes an abstract of each reprint he reads on a 3 x 5" index card and keeps his own file. This provides a ready reference not only for study, but one that can be used after graduation as refresher information or even for research purposes.

As indicated by the above authors, the theoretical education is only as good as its practical application. Yet, without it, the practical techniques are strictly mechanical.

CHARLOTTE M. STREET, New York, U. S. A.:

All phases of theoretical education have been amply described by the above authors. I would merely emphasize that a firm foundation should be acquired in anatomy, histology, basic cytology, endocrinology and the general concepts of the structure and behavior of tumors. Also, a thorough knowledge of normal exfoliative cytology of all body systems is indispensable to the recognition of deviations from the norm, and the basic criteria of malignancy as applied to exfoliated cells must become for the student a subconscious yardstick in evaluating cell changes. Theoretical education in both technique and microscopy, to be effective, must be closely allied with practical application.

DOROTHY B. WELLER, Providence, Rhode Island, U. S. A.:

I agree in general with the program suggested by Grace Durfee and would add to her outline only the following points, which were mentioned by Bickenbach and Soost, and by Maria del Campo: the technique of collecting and processing specimens; the theory of the function of a microscope (in addition to practical training in its care and use).

A knowledge of the types of surgical biopsies which may be performed to confirm a cytological diagnosis and the significance of surgical pathology reports are

valuable to a cytotechnologist, particularly when she has access to these reports for further study of the case and correlation of cytological and histological findings.

## CLOSING REMARKS

**WERNER BICKENBACH and HANS-JÜRGEN SOOST:**

Alice Clark raised the question as to whether or not the general medical pre-education should be the prerequisite for or a part of the theoretical training of the cytotechnologist. This depends greatly on the stage of education with which the future cytotechnologist enters the training program, and which teaching facilities are available. If there were schools for cytotechnologists—which certainly would be the ideal solution—the general medical pre-education could belong to the program of this school. The medical education could be presented in such a concise manner that the important topics for the cytotechnologist could be stressed, and other less important topics omitted. The general medical training could be shortened considerably if the future cytotechnologist already had passed the State Board examination of general medical technology, or if he or she is already an assistant in a doctor's office.

We do not consider it feasible to provide sound training in all phases of cytology within a period of two or three years, if all phases are presented to the trainee at the same time. We consider it more practical to provide the future cytotechnologist first with a knowledge in one particular phase of cytology in which she could be then used as a pre-screener. The other phases would be touched on only in a general orientation program. The first phase in our institution is the teaching of gynecological cytology. One might, however, select another phase. If one is completely familiar with one phase, e.g., gynecological cytology, one will find it relatively easy to make oneself familiar with the cytology of other organs.

Our response to the discussion of Irena Koprowska is by and large contained in the above closing remarks, and also in the closing remarks in the first topic of this Symposium. Certainly the theoretical and practical training must be closely correlated.

**GRACE R. DURFEE:**

I agree with most of the discussants of the program for practical and theoretical training of cytotechnologists with the exception of Miss Atwood of Birmingham, England. Technologists require a suitable academic background and a period of no less than one year of specialized training and practical experience under adequate supervision in an approved school and laboratory. Previous training in other types of laboratory work is extremely helpful but in our experience this should not be considered a prerequisite for training in cytotechnology. The recognition of cytotechnology as a special field places the training and experience on a higher level. Both theoretical and practical training are most essential since one cannot reduce the knowledge of cytology to a mere matter of mechanical techniques.

Mrs. Clark of Chicago is interested in a more precise time schedule in the program of training for cytotechnologists. It is difficult to set up exact schedules which can be applied in every teaching laboratory and which would be generally acceptable. However, a month may be devoted to each of the following aspects of gynecological cytology, namely, normal cytology, inflammations, borderline atypia and malignant changes. One month would be sufficient to cover metastatic carcinomas, effects of radiation on normal and malignant cells and unusual tumors of the female genital tract. The last two months should be devoted to practice screening, review and examination. Theoretical education should occupy about one-fourth of the training period.

**EDMUND SCHÜLLER and MARGARETE PEUTSCH:**

We select our future cytotechnologists exclusively from the ranks of the licensed medical technologists. This eliminates the necessity of the major portion of the required medical pre-education. The theoretical discussions are initially restricted to gynecological cytology and we mention merely analogies to other fields. Those who achieve full experience and knowledge as screeners in gynecological cytology are then introduced to cytology of the respiratory, gastrointestinal and urinary tracts.

# IF THERE WERE A LICENSING EXAMINATION FOR CYTOTECHNOLOGISTS, OF WHAT SHOULD THIS EXAMINATION CONSIST?

RACHEL R. ACHENBACH
Salem, Massachusetts, U.S.A.

I find it impossible to discuss a licensing examination in cytotechnology without mentioning the difficulty imposed by the lack of agreement concerning the role of the cytotechnologist. This role is so complicated by the wide variation in employment opportunities that it seems necessary to consider first the work currently being performed by technicians engaged in cytology.

The minimum job requirements for a trained cytotechnologist include the preparation and staining of specimens and the careful screening of all slides. For the technician working under the active supervision of a cytologist or cytopathologist, this may be the extent of her activities. But in some laboratories, she may also be expected to attend clinics, take part in research projects, or do a considerable amount of teaching.

The cytotechnologist not working under such supervision inevitably acquires the responsibility for the interpretation of ALL specimens. She may work alone for just a group of doctors or a clinic; she may be employed by a pathologist whose time or cytologic proficiency is limited; or she may be successfully self-employed.

Without considering the professional ethics involved, it has been proved that all of these situations CAN be satisfactory from the point of view of service rendered. More than a few "unsupervised" technicians with years of experience in cytology have become accomplished diagnosticians and as consultants are welcomed by the medical staff.

Such situations will exist as long as clinicians are satisfied with the quality of the work performed by non-professional examiners, and I assume that until the services of a professional cytologist are available to every physician, the substitution of cytotechnologists will continue without major protest.

The basic purpose of a license in cytotechnology should be to assure every physician that specimens submitted to a cytology laboratory will be accurately interpreted. Therefore, a license would be of value to the physician only if it were issued to technicians who passed an examination designed to determine their diagnostic ability.

A written examination, however well prepared, cannot test a candidate's diagnostic accuracy. A practical examination, although more difficult to administer, would provide a better indication of the quality of the work of which a candidate is capable. The candidate should be supplied with smears and some clinical information in a series of cases of sufficient number and variety to test her ability to make an accurate diagnosis. And if she were asked to comment on each smear, the examiner could also evaluate her grasp of the clinical implications of the cytological specimen in each case.

In summary, I would propose that licensure of the cytotechnologist state quite clearly the extent of determined ability. If but one examination is to be used, it should be so constructed as to differentiate those technicians whose knowledge and experience enables them to provide satisfactory diagnostic service in unsupervised situations, from the technicians whose qualifications and training should limit them to the role of a screener.

JOSÉ R. DEL SOL
Madrid, Spain

In my opinion a licensing examination should consist of the following: the examination should be divided into two parts, practical and theoretical. The latter having two parts, one written and one oral.

Practical Examination

Screening of 20 slides during a minimum time, giving the hormonal and bacteriological diagnosis and interpretation of malignancy. Ten minutes allowed for each slide, with two rest periods: 15 minutes between the first and the second hour of the examination and 30 minutes between the second and the third hour.

Requirements for passing: All the slides must be interpreted. The following errors are not allowed: Confusing a case of Class III for a Class I; considering a case of Class IV as Class II; 20% error in the interpretation of Trichomonas infestation.

We give less importance to errors in hormonal interpretation, even though it is taken into consideration when giving the final mark.

Theoretical Examination

Answering questions based on the fundamental points of colpocytology and elementary concepts of female genital endocrinology and bacteriology. The first part of the examination is written and lasts two hours. Those who pass are then examined orally. The oral part takes in aspects not covered in the written part and also gives us an idea about the personality of the future cytotechnologist.

A grade of at least 75% in the written test is required for passing.

## CLAUD W. TAYLOR

### Birmingham, England, U.K.

In Britain there are regulations governing the employment of hospital laboratory technologists within the National Health Service. Salary varies according to Grade which depends upon passing examinations held under the auspices of the Institute of Medical Laboratory Technology (I.M.L.T.). A student technician of suitable education is given three years of training in general laboratory methods; an intermediate examination can then be taken. A junior technician who has fulfilled the above qualifications trains (usually for at least two more years) to take a final examination in either histopathology, bacteriology, parasitology, hematology or biochemistry, and then is graded as a technician. On passing a second subject of the final examination the grade of senior technician may be achieved. The examinations, which include written papers and practical work, are conducted by pathologists and technologists. At present there is no examination in cytology. A qualified technician can submit a thesis or dissertation on a subject such as cytology instead of taking a second final subject to become a senior technician.

There is no rank of "Cytotechnologist." A person carrying out this work may be employed as a student technician with no hope of progress unless he or she undertakes to train and pass examinations in other branches of hospital technology. In practice, many take up cytology only when they have already achieved technician status in the other fields of hospital laboratory technology.

Approaches have been made to the I.M.L.T. to institute a final examination in cytology but apparently it is not considered to be comparable in scope with the other final subjects. Cytology may be included as part of the examination in Histopathology but at present only from the aspect of preparation and staining of smears. A period of preliminary general hospital laboratory training has many advantages, but those who desire subsequently to specialize in cytology should be encouraged to do so; under the present regulations they either lose the chance of progressing in their particular field of endeavor and have to learn additional techniques in which they may have no interest.

In University and Research Departments it is often possible to employ technologists on terms not governed by those of hospital laboratories in the National Health Service. Some "cytotechnologists" are employed in this way. However, if they wish to transfer to hospital laboratories they find that, though fully experienced, they may be graded, according to regulations, only as student technicians.

If cytology continues to grow as a laboratory technique, provision will have to be made for some form of examination with recognition of the technologists concerned. If not, a separate licensing body to set up regulations for "Cytotechnology" may come into being. I personally would prefer to see cytology develop as a technical procedure within the general scope of the hospital laboratory rather than as a separate science, but it must have recognition in the same way as the other special subjects. A technologist working in this field, properly trained, should be entitled to expect a progressive future. The I.M.L.T., already established as a body to govern the status of technologists, has a close liaison with pathologists and should be the ideal institution in guiding the future of those engaged in the technique of cytology.

## DISCUSSION

PIERRE HAOUR, C. PASCAL, N. DENAVIT and L. TRONCY, Lyon, Rhône, France:

There does not seem to exist in any country a unique status for cytotechnologists. In many countries two possibilities exist: 1) the cytotechnologist is an individual already trained in laboratory technology who has specialized in the branch of cytology; 2) the cytotechnologist is a person who has experience principally in cytology, gained sometimes after many years of working with a cytologist. For these already well-trained cytotechnologists no examination should be required. However, technicians who now begin studying cytology, should be required to take an examination. This examination in our opinion could be divided into two parts:

a. examination on general laboratory techniques (theoretical and practical);
b. examination on cytological techniques (theoretical and practical).

The practical part should consist of special screening tests performed as proposed in the discussions elsewhere in this Symposium.

Rachel Achenbach has rightly pointed out that the role of the cytotechnologist is not yet well-defined and of course the nature of the licensing examination will depend greatly on this future definition of the role of a cytotechnologist. This is an important point to discuss before establishing a licensing examination, as well as any program of training.

IRENA KOPROWSKA, Philadelphia, Pennsylvania, U. S. A.:

A licensing examination will probably be taken by a progressively larger number of cytotechnologists, especially in this country. A body of examiners, composed primarily of cytopathologists and possibly including consulting cytotechnologists, will ultimately agree upon the details of a comprehensive practical and theoretical test.

It is logical that a practical examination should include an evaluation of cytologic findings in selected smears and also, if possible, testing of technical laboratory proficiency, such as skill in preparation of sputum smears. A theoretical examination could be written or oral or both.

A licensing examination should provide satisfactory means of finding out if candidates have a sufficient understanding of important problems in all aspects of Exfoliative Cytology, a sound approach to the evaluation of cellular findings in smears, and familiarity with basic technical laboratory procedures. Licensed cytotechnologists should be prepared to act as dependable screeners. It does not appear desirable to plan for preparation of future cytotechnologists as ultimate cancer diagnosticians. Consequently, one standard type of licensure for the cytotechnologist would be adequate.

Nevertheless, since the number of competent cytopathologists is still inadequate, it would be unwise to underestimate the potential value of the experience, educational background and intelligence of several outstanding cytotechnologists and decline use of their time-tested

competent independent services. Issuing special certificates of recognition to selected, well-established non-medical cytodiagnosticians upon the recommendation of an ad hoc appointed committee of the Inter-Society Cytology Council may be a worthwhile matter to consider. I would prefer allotting such a recognition to deserving old-timers than to face the existence of two types of licenses as suggested by Rachel Achenbach. Requesting permission of graduate schools to recognize that Exfoliative Cytology may be selected as a major by M.S. (Master of Science) or Ph.D. (Doctor of Philosophy) degree candidates, if granted, may open new sources of gratification to ambitious, more-recently-trained cytotechnologists. Positions of instructors in cytotechnology schools or admission to membership in research teams could be reserved for individuals in this category. Their privileged positions would be recognized, but the danger of placing the responsibility for medical diagnoses with a non-medical personnel would be eliminated.

In reply to Taylor, I may briefly say that I agree fully with the desirability of providing recognition for cytotechnologists. I am afraid that this will take some time in Great Britain because of an even greater shortage of cytopathologists there than in the United States of America. It would be through cytopathologists that the National Health Service could be sufficiently influenced to provide recognition for the hospital cytotechnologist. Once this is accomplished, there would also be a greater chance to attract more suitable candidates into the field of cytotechnology.

Del Sol's proposed examination is intended for testing the knowledge in the field of gynecologic cytotechnology only, and as such, would be of a limited applicability. Also, I would not consider all cases reported as suspicious to be equally easy for the cytotechnologist to recognize. Consequently, it may not be necessary to disqualify a candidate who would fail to evaluate correctly one of twenty cases, but who would exhibit sound judgment and thoroughness in the evaluation of the remaining 19 cases.

F. A. LANGLEY, Manchester, England, U. K.:

It is difficult to discuss licensing examinations apart from the type of laboratory organization peculiar to an individual country. I agree with Taylor that cytology should develop as a technical procedure within the general scope of the hospital laboratory. Two difficulties seem, however, to arise. First, at present there is not a great demand for cytotechnologists in Great Britain and thus no real pressure can be brought on the I.M.L.T. for proper recognition of this specialty. In part this constitutes a vicious circle for in the absence of qualified cytotechnologists many laboratories cannot undertake cytological investigations. Second, the I.M.L.T. lays great stress in its examinations on technical procedures and does not generally encourage technologists to interpret their results (that is the pathologist's work). In cytology there is relatively little technique to learn but much about interpretation. This raises a difficult and delicate issue. I agree with del Sol's outline for an examination, but would add the following: In the practical examination the student should prepare and stain a number of smears himself. The theoretical examination should include questions requiring an elementary knowledge of both general pathology (tumors and inflammation) and the special pathology of the female genital tract.

CHARLOTTE M. STREET, New York, New York, U. S. A.:

I agree with Rachel Achenbach that a practical examination is a better test than a written examination as to the quality of work of which a candidate is capable. However, a written examination is of advantage in assessing a cytotechnologist's grasp of the basic concepts of exfoliative cytology which are, after all, important.

Whether the examination is only a practical one or includes written and oral sections, it should test thoroughly the candidate's knowledge of all applications of exfoliative cytology and not be limited to gynecological cytology. The practical examination should include a sufficient number of well chosen slides to prove his ability as an accurate screener, and credit should be deducted for false positive as well as false negative interpretations. Regarding written examinations, I feel that the present trend toward the multiple choice or true-false type leaves much to be desired as there are too many exceptions to the rule in exfoliative cytology, and furthermore such an examination is not always truly indicative of a candidate's knowledge.

If the licensing of cytotechnologists would place the official stamp of approval on their working without proper supervision, then the standards for such licensing must be set very high. Even then, as a safeguard to the patient, consultation with a qualified professional cytologist or cytopathologist, in cases of positive findings, should be made mandatory.

## CLOSING REMARKS

RACHEL ACHENBACH:

I previously stressed the importance of a practical type of examination because I feel that a license held by a cytotechnologist will be interpreted by clinicians as assuring competence in the interpretation of cytologic material, particularly where the services of a cytologist are unavailable. By doing so, I certainly did not mean to underemphasize the importance of a thorough knowledge of basic concepts.

As an "old" cytotechnologist, I hate to argue against offering a license without examination to those with seniority, as suggested by Haour, et al. in their discussion, but I seriously question the fairness of the proposal to the cytotechnologists more recently trained. I have a similar objection to Dr. Koprowska's suggestion of a special certificate of recognition, unless it were strictly honorary and did not imply certification of any kind. In most teaching laboratories the training in exfoliative cytology now being offered is much more comprehensive than that offered ten or fifteen years ago, and even with the advantage of long experience, most of us old-timers who have attempted to keep up with the newer developments have had to make a special effort to do so.

I know it would be easiest, and perhaps it would be best, to plan a licensing examination to determine only the cytotechnologists' ability in laboratory techniques and the preliminary screening of slides. But I think we must keep in mind that for some time to come there will be far too few trained cytologists to relieve all cytotechnologists of the responsibility for the final diagnosis, even a diagnosis of malignancy. And it is the clinician who is most concerned who needs some means of determining the dependability of the cytologic service available to him.

CLAUD TAYLOR:

The various discussants emphasize the differences that exist regarding the status of cytology in different countries. In some it should be possible to organize training and a qualifying examination in cytology; in others it will be necessary to consider cytological techniques only as part of general laboratory technological training. In all cases it is the duty of those in charge to continue to press for recognition of all engaged in cytotechnological procedures.

# SHOULD THE CYTOTECHNOLOGIST BE TRAINED TO TAKE THE SMEARS HIMSELF?

MARIO S. BLANCO de del CAMPO
Palo Alto, California, U.S.A.

It is to his advantage as well as that of the laboratory if the cytotechnician knows how to collect smears. Dealing directly with patients strengthens his sense of responsibility and emphasizes for him the human significance of his work. Learning the pitfalls of specimen collection makes him able to advise contributors who are submitting poor specimens on ways of doing better. And finally, if the technician collects the specimen himself, he is much better able to ensure optimal quality in the finished preparation.

BIRGITTA FREDRIKSON
Gothenburg, Sweden

It is of great importance for the diagnostic accuracy that the smears are correctly taken. I believe that the cytotechnologist should be trained to take the smears himself in order to obtain uniformly taken and fixed smears. Now and then the laboratory is requested to take smears on wards where vaginal smears are not routinely taken. It has been the courtesy of the lab to send the cytotechnologist to take the smears rather than to instruct the nurses or the doctor on the ward. Furthermore these wards are not provided with the necessary equipment.

RUTH M. GRAHAM
Buffalo, New York, U.S.A.

I feel it is essential that the cytotechnologists have some training in taking specimens, whether or not they will be required to obtain them in their permanent position.

It is extremely difficult, if not impossible, to instruct others in the method if one is not fully familiar with all the difficulties that may be encountered. Technicians are often asked how the smear should be obtained and fixed. Not only should they be required to take the specimens but to screen the smears they have taken. By correlating the microscopic picture with his technique the student can learn to recognize drying of the cells before fixation, inadequate distribution of the material on the slide, and other factors which contribute to a poor technical preparation.

It is my impression that frequently too little attention is paid to the technical aspects of the cytologic diagnosis of cancer. Technicians have a period of training which is predominantly microscopic work. Accuracy of diagnosis is directly related to the adequacy of the smear examined—and this means not only adequate material, but properly prepared material. The cytotechnologist should be taught to recognize the poorly prepared slide and the factors which contributed to the poor preparation. This can best be accomplished by teaching the cytotechnologist to take the smears.

## DISCUSSION

**WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany:**

We agree with the above authors that training of the cytotechnologists in preparation of the specimens should be part of the training program. The cytotechnologist has to know from personal experience how the smears are prepared. Only then will he be able to give pertinent information to the inquiries of physicians. And only then will he be able to recognize and evaluate errors that occur due to faulty preparation technique.

**HANS-WERNER BOSCHANN, Berlin, Germany:**

The proper preparation of the smear from the correct site is the most important prerequisite for the reliable hormonal evaluation. The smear preparation is the task of the physician. However, I agree with the above authors that the cytotechnologist should be acquainted with the following:

1. the preparation of smears from the lateral vaginal wall (for hormonal evaluation) and
2. the preparation of smears from the posterior vaginal fornix and the ecto- and endocervix (for cancer detection).

The technologist should be acquainted with the above in order to be able to recognize if the preparation has been done properly and in order to be able to inform the physician submitting the smears of the proper preparation technique.

Obtaining cytological material from the uterine cavity (for diagnosis of carcinoma of the uterine corpus), from the cul de sac and from punctures in cases of adnexal tumors, ascites and circumscribed tumors (for instance lymph nodes, tumors of the Bartholin glands and of the vaginal wall) are permitted under sterile conditions a priori only by the gynecologically trained physician.

ALICE DARGAN CLARK, Chicago, Illinois, U. S. A.:

All the above authors seem to agree that the taking of smears is or should be an integral part of the training of the cytotechnologist. They have clearly pointed out the numerous values of such training.

Now, some specimens are routinely prepared and fixed outside the laboratory, while others (fluids, urines, bronchial washings, etc.) are usually sent to the laboratory for more involved preparation. Whether or not the cytotechnologist would be required to make routine smears outside the laboratory probably depends upon the practicability in the particular situation. However, I am in complete agreement with all of the authors as to the necessity for some such experience during the training period for the reasons they mention.

MARCEL GAUDEFROY, Lille, Nord, France:

For the human significance of his work and the optimal quality of the specimens, I believe it is a real advantage if the cytotechnologist, and the cytologist himself, personally collects the cytological material. I agree completely with the assertions of the above authors. I happen to be a gynecologist, a gynecological-pathologist, and a cytologist. In my laboratory, my assistant and I read the smears prepared by myself and smears from other clinics, hospitals, or individual physicians and gynecologists. As a general rule, the smears collected by myself are more uniform, adequate, and easier to evaluate than the others. However, it is impossible in a large cytology center to have all smears obtained by the cytotechnologists themselves. It seems to me that the cytotechnologists must have training in the preparation of specimens, and all physicians, at least the gynecologists, must have some training in reading cytological smears in order to understand the difficulties of interpretation resulting from inadequate collection and fixation of the specimens.

OLAF T. MESSELT, Oslo, Norway:

The answer will depend on the routine in the hospital in question.

In the Norwegian Radium Hospital the vaginal smears from in-patients and patients consulting our out-patient clinic are taken by gynecologists in connection with their examination of the patients.

Personally I regard smears made from scrapings from the external os as more valuable than aspiration material from the posterior vaginal fornix, and accordingly such smears are taken in all cases. In my opinion it would be an insult to the doctors and seem ridiculous if I should request the gynecologist to step aside during his examination of the patient to allow my cytotechnologists, who have no medical education and who do not know how to insert a speculum or to find the portio, to take the smears. I find it much easier to teach a gynecologist the proper way to collect the material and make satisfactory smears. The bulk of vaginal smears in our laboratory comes from general practitioners, gynecologists and hospitals all over the country, and in all these cases the only thing I can do is to give advice and point out possible reasons for poor smears.

In cases where unfixed material is being sent to the laboratory, for instance sputum, exudates, etc., the technicians are, of course, taught how to handle the material and prepare it properly.

HANNAH PETERS, Copenhagen, Denmark:

I agree that the cytotechnologist should know (1) how to obtain the specimen from which the smear is prepared and (2) how to make the smear from the specimen. However, in many cases his knowledge of how to obtain the specimen will have to remain theoretical (lung, prostate, pleural, peritoneal, uterine, etc.), yet in order to know the causes and remedies for unsatisfactory smears, he must become well acquainted with the technique of preparation of specimens.

LUIS MONTALVO-RUIZ, Madrid, Spain:

In principle we believe that the smears should be taken by gynecologists, but we do not see any negative reason why the cytotechnologist should not be trained to do it. One should have a special room annexed to the cytology laboratory for this purpose with all the necessary materials for taking smears, such as gynecological table, speculum, slides, Papanicolaou pipette, Ayre spatula, cotton swabs, alcohol-ether fixative bottles, good illumination, etc. The Gynecological Clinic staff should supervise the collection of the smears by the cytotechnologist.

PETER STOLL, Heidelberg, Germany:

Correct preparation is an important prerequisite for correct cytological diagnosis. We share the viewpoint that the crucial problem is not so much difficulty in the evaluation of the material submitted for examination but in proper preparation technique. For this reason the cytotechnologist should master the methods of smear preparation. In gynecological cytodiagnosis he has to know the difference between the direct and indirect smear preparation, since the cytological patterns are different. He must be able to decide whether or not the smear can be evaluated. This will depend upon careful smear preparation and also upon the further work-up. His interest will increase when one brings him into contact with the patient and lets him participate in clinical considerations.

In this regard I agree with the discussants, especially with Ruth Graham. On the other hand, we do not deem it correct to have the cytotechnologist take all the smears. For us, cytology is a clinical diagnostic method, which has to be evaluated together with the clinical findings. As a rule, we take the smear during the gynecological examination, in the course of which the cervix is visualized by means of a speculum. Only under these conditions is it certain that the indirect smear is taken from the anterior vaginal fornix and that in the direct preparation from the ectocervical surface and the endocervical canal the correct site is reached. We rarely do blind aspirations with the pipette; only in those cases where the first regular smear was normal and a series of smears for the evaluation of the functional course has to be done, the inspection of the ectocervix has to be reserved for the physician, since only he may decide whether or not there is a grossly suspicious lesion present—because of his experience. I base my viewpoint on the following two observations:

1. In the presence of an advanced cervical carcinoma, which is already visible to the naked eye and is ulcerating on the surface and has an inflammatory reaction present, the indirect smear, secured by means of the aspiration pipette, could possibly result in a false negative report, since occasionally merely cellular debris, leukocytes and red blood cells are aspirated and carcinoma cells are not discernible. In this case, if the cytotech-

nologist would take the smear by means of the aspiration pipette and the cytological reading would be negative or only suspicious, then the confidence in the cytological method would be harmed. Our investigations show that in the presence of a manifest cervical carcinoma in its ulcerated stage, more than 30% of the smears prepared by blind aspiration have resulted in "false negative" reports. The cytotechnologist cannot assume this responsibility.

2. In cases of a cervical erythroplakia the direct preparation of the specimen from the site of epithelial changes is important for the diagnosis. If the physician sees such an alteration then he should take the smear from this site and must not restrict himself to the preparation of the specimen from the lateral vaginal wall or to the aspiration technique with the pipette.

If one shares the opinion that the cytological smear preparation is a procedure of high responsibility, then the preparation as a general rule has to be done by the physician who assumes responsibility for his patients. There exists a relation based on confidence and also a contractual agreement between patient and doctor which the former has selected by his own choice. He entrusts himself to this doctor's care and wants all diagnostic and therapeutic procedures done by him. For this reason it would not be legal in our country to give to a technical aid the responsibility of preparing the vaginal smears. The responsibility of the laboratory in the evaluation of the smears is already big enough. Without doubt medical situations are different in Germany and in the United States. In Heidelberg we share the opinion that young physicians should be acquainted with cytology as a clinical diagnostic method. As a University Department we are a teaching institute for young physicians and specialists, who later are supposed to employ the methods when working in their own offices. For this reason we have a rule that the physician himself has to take the direct and indirect smears, during the gynecological examination. He has to study immediately a fresh smear under the phase contrast microscope and has to put down his findings in writing. The additional smears prepared for the cytology laboratory have to be fixed immediately under his supervision. This latter is done by the office or the floor nurse. On the same day the same physician has to prescreen the smears prepared by himself, which in the meantime have been stained in the cytology laboratory, and to write down his diagnosis. His findings are checked by an experienced cytologist.

In this way we have acquainted all the residents and assistants of the Department with the practice of cytodiagnosis. Each physician can compare his cytological reading with his clinical findings and attains thereby a better picture of the condition of his patient. With 230 beds, we have today 16 assistants or residents. Three of these hospital residents are able to give cytological diagnosis by themselves because they have been working in cytology for more than two years. Besides myself, these residents or assistants are asked for advice by their younger and less experienced colleagues in the evaluation of suspicious or inconclusive cytological patterns. We have employed for the cytological diagnosis only physicians; the cytotechnologist is merely responsible for the technical processing of the smear. As a matter of fact we do not have any cytotechnologists in the American sense, since our technicians are engaged, in addition to the processing of the cytological material, also in the processing of the histological specimens. However, on the grounds of their experience they have acquired a certain knowledge in this matter and are able to give to the student suggestions and may be of some help, but they do not carry any diagnostic responsibility of their own.

Besides the above considerations, the personnel situation in Germany is unique in so far as there is an abundance of medical doctors but a shortage of technical personnel. Our laboratory employs seven technicians: 3 for morphology (histology, cytology and hematology), 2 for the special coagulation laboratory, 1 for the photographic laboratory, and 1 for urine analysis and pregnancy tests. For a comparison, our Hospital has 230 beds and the number of patients cared for in our Out-Patient Department is 70 per day. For these reasons the technical personnel is mainly employed in the technical preparation of the smears, whereas the diagnosis itself is done by physicians.

Summarizing, I may say that it is entirely correct to introduce the cytotechnologist to the preparation technique of smears in order to give him an insight into the problems of the preparation of the specimens. The preparation itself, however, and the final diagnostic evaluation should be done only by the physician.

CHARLOTTE M. STREET, New York, New York, U.S.A.:

I agree with the apparently unanimous opinion of the authors that there are advantages in having the cytotechnologist trained to take smears himself.

I should like to add that if the cytotechnologist is to be trained to take cervical or endocervical smears in addition to vaginal smears, instruction should be given by a physician who can show him the proper procedure for inserting the speculum, the use of the various instruments employed in obtaining these secretions, and any anatomical features that he should know.

The cytotechnologist should not be expected to obtain endometrial smears as he lacks the necessary medical training and experience for such a procedure.

## CLOSING REMARKS

MARIA S. BLANCO de del CAMPO:

I appreciate the above comments and wish to reassure Messelt and Stoll, respectively, that knowledge of smear collection by the cytotechnologist does not imply vesting him with the responsibilities that only the physician should have in the same fashion that his role as a screener does not assume the functions of the pathologist. If the law forbidding the insertion of a speculum by nonphysicians were revised to allow the cytotechnologist to use the speculum, he would be of greater help to the profession as a smear collector, e.g., collecting cervical smears from an asymptomatic population in a community interested in determining the incidence of cancer and/or the early detection of cancer.

# SHOULD THE CYTOTECHNOLOGIST BE TRAINED IN HORMONAL AND MICROBIOLOGICAL EVALUATION OF THE VAGINAL SMEARS?

JEAN A. de BRUX
Paris, France

The answer appears so evident that the question seems superfluous. One cannot be qualified as a cytotechnologist if one is not capable of evaluating the hormonal status of a smear.

This presupposes, as I have pointed out in a preceding topic, an acquaintance with the ideas of endocrinology, histophysiology and cytophysiology. The comparison of endometrial slides at the different stages should serve as a means of control. Moreover, frequent comparisons of the vaginal hormonal status and the endometrial state are very fruitful, for by this means one may 1) reconsider certain percentages of pyknosis and 2) understand seemingly unexplicable apparent anomalies among smears during a cycle which is nevertheless regular.

Furthermore, very difficult hormonal problems such as that of the adrenal action on the cellular morphology of the vagina cannot be attacked without a sound knowledge of endocrinology and a close collaboration between the cytologist and the technician, the latter calling the attention of the cytologist to the changes encountered and asking for explanations.

But, after such an enumeration, we may parody Beaumarchais in "Figaro," and say, "With all the qualities expected of a cytotechnician, how many doctors would be worthy to become one?"

BIRGITTA FREDRIKSON
Gothenburg, Sweden

An intimate knowledge of the hormonal status and the microbiological flora is of great importance for the diagnostic accuracy. The cytotechnologist has to be acquainted with the varying cytological pictures corresponding to different hormonal situations. A certain hormonal picture which per se is not pathologic might be pathologic when the clinical facts and circumstances are considered, such as abnormal estrogen stimulation when ovarian activity should have ceased or signs of ovarian inactivity during the reproductive period. The screening cytotechnologist must be able to recognize a cytological picture which is pathologic when clinical circumstances are considered.

EMMERICH von HAAM
Columbus, Ohio, U.S.A.

My answer to this question is, "Yes," and the reasons for this answer are as follows:

To report only positive and negative vaginal smears becomes boring to an intelligent and well-trained cytotechnologist, and the small number of positive reports which will come from such a laboratory will often make the clinician lose interest in the entire diagnostic procedure. The vaginal smear includes so much more interesting material with regard to the patient that it seems a pity not to use all the information one can get, particularly since this information is often as much appreciated by the referring physician as the report concerning the presence or absence of malignant or suspicious cells. In addition, all cytotechnologists in our course are fully trained with respect to hormonal and microbiological evaluation and should utilize this training if they are to retain their knowledge. However, in order to give a hormonal evaluation, cytotechnologists should be supplied with at least two data: the age of the patient and the date of the last menstruation. With regard to microbiological evaluation, we do train our cytotechnologists to recognize and differentiate the various types of bacterial flora as can be demonstrated by the Papanicolaou technique.

In both instances the cytotechnologists are trained to differentiate the normal from the abnormal, but they are not trained to interpret abnormal findings in terms of a specific disease condition present in the patient.

PATRICIA KELLY

San Francisco, California, U.S.A.

When the work is properly supervised by a doctor trained in cytology, the cytotechnologist should, in my opinion, be taught to report the preliminary findings in hormonal and microbiological evaluation. Chance findings in screening may have an important significance, and for this reason I believe such training is imperative.

Many times a smear which is negative for malignancy may have changes that should be brought to the attention of the cytologist. If, for example, a routine smear were screened on a patient with a six-month pregnancy and the cells showed no evidence of malignancy, a cytotechnologist, untrained in hormonal evaluation, would be correct in writing a preliminary report of "Negative." However, this same smear may contain a high percentage of eosinophilic (karyopyknotic) superficial cells, a marked lack of cohesiveness in squamous cells, phagocytic histiocytes and a number of red blood corpuscles—all of which are benign but of great significance in this case. I believe, it would be of far greater value if, in such cases, cytotechnologists were trained to dot representative cells from each cell type, number each dot and write a complete report of the findings, indicating the cells that have been dotted and numbered. A Karyopyknotic Index reflecting the smear as a whole, not just a small portion of the smear, should be included in this report.

This method of reporting applies very well to other types of normal and microbiological evaluation. It is true, of course, that all smears are reviewed and diagnosed by a cytologist. But a cytologist has great demands on his time, and, I believe, an important role of the cytotechnologist is to accurately anticipate what should be noted for the doctor's consideration.

## DISCUSSION

RACHEL R. ACHENBACH, Salem, Massachusetts, U. S. A.:

More recognition should be given to the fact that in many laboratories, specimens considered to be unremarkable by the technician screener are not examined further by anyone. Under such circumstances, if the clinician is to receive the benefit of hormonal and microbiological evaluations in his "negative" smears, it would seem imperative that the screening cytotechnologist be thoroughly trained in these areas.

J. ERNEST AYRE, Miami, Florida, U. S. A.:

The cytotechnologist should certainly receive training in hormonal study, and microbiological studies, while desirable, would seem to be more essential for the screener than for the cytologist. However, as always, the more widely experienced the cytotechnologist, the better the diagnostic results should be.

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany:

We agree with the above authors that the cytotechnologist should be trained in the hormonal and microbiological evaluation of smears. For without a profound knowledge of the hormonal evaluation of smears and of the microbiological conditions of the vagina, a rational evaluation of smears for the diagnosis of carcinoma will not be possible. Moreover, we agree with von Haam that it soon would become too boring for the cytotechnologist merely to search for atypical cells.

In our opinion, the microbiological status, as far as can be carried out with the slide stained accor ing to Papanicolaou, may be evaluated in almost all case by the cytotechnologist without help from the cytologist. For the hormonal evaluations this is not so unequivocall valid, for the cytological pattern is only one part of the diagnosis. The hormonal evaluation proper arises from the combination of cytological pattern, history and clinical picture, the evaluation of which requires profound knowledge of gynecological endocrinology. Without this knowledge one will not be able to distinguish pathological from normal cytological patterns and the pre-screening of cytological smears in regard to the hormonal evaluation is possible only to a limited degree. The intelligent cytotechnologist, who is interested in clinical problems, will be able, after a certain time of practice, to relieve the cytologist of many of these cases. However, in our opinion it would be requiring too much to consider, as th goal of a two year training course, such an extensive knowledge in clinical endocrinology as is necessary for the hormonal evaluation of cytological specimens.

HANS-WERNER BOSCHANN, Berlin, Germany:

I agree with the above authors that each cytotec nologist should be trained in microbiological and hormon evaluation of cytological smears. The reasons have been brought out convincingly by the above authors. The cytotechnologist should be able to answer the following questions from the smear:

1. Does the cytological pattern correspond to the age of the patient?
2. Does the cytological pattern correspond to the phase of the menstrual cycle of the patient?
3. Does the smear pattern resemble any of the following? a. an atrophic smear type, b. an intermediate proliferation of androgenic type, or of progestational type, or c. a highly proliferated smear type of the estrogenic type?
4. Does the degree of proliferation seem constant throughout the smear or are there appreciable fluctuations?
5. Does the smear pattern appear to be compatible with pregnancy, and if so, are we dealing with a normal pregnancy, or with a disturbed pregnancy?
6. Does the hormonal pattern seem to be simulated by inflammatory or bacterial influences? Are, for instance, coccoid bacteria or Trichomonas causing a pseudo-estrogen effect, i.e., false eosinophilia and pseudopyknosis? Or, is an estrogenic effect obscured by the presence of Döderlein bacilli which cytolyze the growing epithelial cells in their intermediate stages?

In difficult questions the cytotechnologist has to restrain himself to the mere description of the patterns found. The diagnosis then has to be completed by the cytologist. The latter, therefore, has to be very well acquainted with gynecological endocrinology. He has to interpret the findings in a way that enables the clinician to draw from it clinical conclusions, which could not be drawn from a description of the cytological pattern alone

ALICE D. CLARK, Chicago, Illinois, U. S. A.:

I am glad that all of the above authors seem to agree on the necessity of training in the hormonal and microbiological fields for the cytotechnologists. However, each seems to vary somewhat as to the extent to which the cytotechnologist should be trained in these areas of study, from "acquaintance," "preliminary re-

port," "sound knowledge" to "fully trained." Could the above authors be more specific as to the educational background of the training? There also seems to be a difference of opinion as to what degree of interpretation should be permitted the cytotechnologist in the case of the abnormal hormonal finding (pathological).

If the cytotechnologist has sound training in endocrinology, accomplished by preliminary theoretical training accompanied by practical experience in both histophysiology and cytophysiology, as pointed out by de Brux, does everyone feel it would be limiting to the cytotechnologist to give only a differentiation of the normal from the abnormal, without going on to suggest what might be the cause of the variance? This abnormal finding would necessarily be discussed with the cytologist before the report was sent out from the laboratory as a final diagnosis. However, to paraphrase von Haam, "to report only normal or abnormal hormonal conditions becomes boring to an intelligent and well-trained cytotechnologist."

LUIS MONTALVO-RUIZ, Madrid, Spain:

The question has two parts. If the cytotechnologist should be trained in hormonal readings, I answer "yes," in agreeing with von Haam and with de Brux, but with the condition pointed out by Patricia Kelly: that a competent cytologist supervise the interpretation.

With regard to the second part of the question concerning the microbiological evaluation by the cytotechnologist, I am of the opinion that if only the Papanicolaou or Shorr stains are used the microbiological evaluation would be always inaccurate. A true microbiological evaluation requires special study (Gram stain, cultures, etc.), which is beyond the cytotechnological training. However, the cytotechnologist should be trained to roughly interpret the bacteriological status of the smear, in other words to identify the obvious elements that the Papanicolaou stain permits, such as Doderlein bacilli, trichomonads and coccoid bacteria without determining the genus.

JOSÉ R. DEL SOL, Madrid, Spain:

We require that our cytotechnologists be capable of making proper hormonal and microbiological evaluations:

a. In our department all cytological reports are accompanied by a hormonal evaluation. This facilitates the clinicians' work.
b. There exist several cytological concepts that cannot be separated from a microbiological point of view, for example, dyskaryosis.
c. For economical reasons, more extensive microbiological investigations are made only in doubtful cases.
d. A high educational level is desirable for the future cytotechnologist. We believe that the most common findings under the microscope must be explained to the cytotechnologist in order for him to obtain more exact criteria for evaluation.

## CLOSING REMARKS

PATRICIA KELLY, San Francisco, California, U. S. A.:

The interesting papers of the discussants indicate that the climate of opinion is in favor of training cytotechnologists in hormonal and microbiological evaluation of the vaginal smear. This conclusion suggests many new questions. Unfortunately, the detailed answers to some of these questions lie beyond the scope of this topic.

I agree completely with del Sol that all cytological reports should be accompanied by a hormonal and, when applicable, a microbiological evaluation. The report should include answers to the questions which are so well stated by Boschann. However, a complete microbiological evaluation requires special preparations, as has been pointed out by Montalvo-Ruiz.

"Preliminary report" does not in any way refer to the degree of training in cyto-hormonal evaluation. It is my opinion that cytotechnologists should be "fully trained" in this aspect of cytology, but whether the technician's report is "preliminary" or "final" depends on the policy of the laboratory in which she is employed. In cases such as the one I cited above, I believe the cytotechnologist's report should "suggest what might be the cause of the variance" but the final diagnosis should be made by a cytologist. However, although I feel that the work should be supervised, I wholly agree that cytology would become completely boring if the technician were not permitted to actively participate in the deeper knowledge and stimulating research which the field offers.

# WHAT IS THE AVERAGE SCREENING TIME FOR SMEARS FROM THE FEMALE GENITAL TRACT, THE RESPIRATORY TRACT, THE GASTROINTESTINAL TRACT AND THE URINARY TRACT?

MARIA S. BLANCO de del CAMPO
Palo Alto, California, U.S.A.

Average screening time for smears from any of these sources would be at least six minutes per slide for an experienced cytotechnician, i.e., one with more than two years of active screening experience whose sole responsibility is to segregate normals from abnormals and who is discriminating in making dots in the atypical smears. Such a technician should be able to screen as many as 80 slides or 40 cases with two smears per patient in an eight hour working day when other routine functions of the laboratory do not require his attention. If they do, he may average as few as 40 slides or 20 cases when a vaginal and cervical specimen is furnished per patient. The volume of slides screened will depend, therefore, on the amount of time spent in other activities of the laboratory. Various atypical morphological features such as an infectious pattern in absence of causative organism, atypical features probably benign in nature, hormonal abnormalities, atypia that may be attributed to pregnancy, severe cervicitis, curettage, etc., or suspicious items of history such as post-menopausal bleeding may increase the average screening time per slide to from 10 to 20 minutes.

BIRGITTA FREDRIKSON
Gothenburg, Sweden

My experience is entirely limited to smears from the female genital tract and from mammary secretion. I think it is impossible to give an average screening time as it varies from case to case. If it is a smear from a woman without gynecological disorders, it does not take long to give a negative report. Smears from patients with a cervical cancer might only take a minute to diagnose. On the contrary other smears are very time-consuming, e.g., smears from patients with gynecological disorders where you often have an abundance of leukocytes, presence of blood and perhaps cytolysis and bacteria causing nuclear changes in the cells. Smears from patients treated with radium and x-rays for cervical cancer are very easy to screen as long as the patient is healed but might be very difficult when the question arises whether she has a recurrence or radiation reaction. As will be evident from another topic in this Symposium, I find it impossible to give an average screening time, as it can vary from a few seconds to one or two hours, depending on the aim of the diagnosis.

HOWARD L. RICHARDSON
Seattle, Washington, U.S.A.

In order to answer this question, many factors must be taken into consideration, such as laboratory organization, previous training of the cytotechnologists and pathologists, relationship of the pathologists to the cytotechnologists, collection of specimens and number of secretarial duties performed by the technologists. A cytotechnologist relieved of secretarial duties will have considerably more time for screening.

All cytology laboratories do not operate under a pathologist or pathologists. I am not qualified to evaluate laboratories operating under special services.

Elsewhere in this Symposium, a chart on the case load of the Strang Cytology Laboratory in New York City is compared with the case load of the Cytology Laboratory in Seattle, organized under pathologists. Using a teamwork system of pathologists and cytotechnologists, it was found that one well-trained technician could screen approximately 24 to 30 female genital cases per day, or 48 to 60 slides (an average of 8 to 10 minutes per slide). In material from the respiratory tract (i.e., sputum, bronchial washings), both cytology and cell block preparations are routine (1,2). This procedure shortens the cytologic screening time to about six minutes. Atypical cell clusters or positive cases may require 20 to 30 minutes per case. The scanty nature of the material from the uri-

nary tract may increase the screening time also.

The histologic preparation of the cell block is reviewed by the pathologist in approximately three minutes. A correlation of the cytology with the histology increases the diagnostic accuracy (3).

In material removed from the gastrointestinal tract, a cell block and cytological preparation are made and treated in exactly the same manner (4). Cytologic preparations are given preference, and cell blocks are made from the residue.

Bibliography


1. Hunter, W. C. and Richardson, H. L.: Surg., Gynec. & Obst. 85:275, 1947.
2. Richardson, H. L., Hunter, W. C., Conklin, W. S. and Peterson, A. B.: Am. J. Clin. Path. 19:323, 1949.
3. Richardson, H. L., Koss, L. G. and Simon, T. R.: Cancer 8:948, 1955.
4. Richardson, H. L., Queen, F. B. and Bishop, F. H.: Am. J. Clin. Path. 19:328, 1949.


## DISCUSSION

ARTURO A. ARRIGHI and GUILLERMO TERZANO, Buenos Aires, Argentina:

We agree with the above authors that in screening time much depends on the type of smears, the ability of the screener, the difficulty of each case, and also the facilities for working. Dividing the number of slides examined by the number of hours spent in front of the microscope, we found that for ourselves four to five minutes is the average time for each slide. For the beginners and the cytotechnologists it is about 10 to 15 minutes. Some cases can be diagnosed in a few seconds, if one has the good luck of finding a group of obvious cancer cells in the first field seen, but there are cases in which 20 minutes or more is not time enough to make a negative or positive decision. It must be said that to screen routine negative smears could easily become a tiresome job with all its peril. Several negative cases in a row may annoy or confuse the observer. To avoid this, microscopic work should be recommended with regular free intervals for relaxation. It is rather difficult for us, based on the experience in our laboratory, to attempt to establish actual standards in screening time.

J. ERNEST AYRE, Miami, Florida, U. S. A.:

Gynecological smears may be more readily broken down into average screening periods, while specimens from other systems are studied individually for quite variable periods of time. Cervical smears or scrapings from charity clinics with patients of low economic classes have larger volumes of positive and suspicious material, requiring longer periods of study than smears from normal groups of women. In the latter, a well-trained screener can handle 40-50 cases per day, while in clinic groups this number is likely to be 20-30 cases per day.

RUTH M. GRAHAM, Buffalo, New York, U. S. A.:

Because the question of the average length of time for screening a slide frequently arises, several years ago I determined the average length of time in my own laboratory. On an ordinary working day, the number of slides screened in a certain length of time was tabulated. The average time per slide was five minutes. The slides were routine and included all types of cytologic material, including sputa, bronchial aspirations, gastric secretions, fluids and urines.

I should point out that the screening was done by experienced technicians, their years in the field varying from eight to three. The experienced technician will screen much faster than the newcomer in the field. Also, this level of efficiency was for a four hour period. It is unlikely that this level could be maintained for an entire eight hour day. Extremely high figures are sometimes quoted for the number of slides a technician can screen in a day. I suspect that these are arrived at by multiplying the number of slides screened in an hour by eight. This is fallacious. I do not think that the high level of attention required in accurate screening can be maintained for an entire day. For that reason, in my own laboratory screening is done in the afternoon. The morning hours are occupied with such tasks as staining, preparing specimens, taking specimens and some record work. Emphasis on speed in screening should never replace emphasis on accuracy.

ELIZABETH H. GRAY, Ottawa, Ontario, Canada:

We quite agree with Birgitta Fredrikson that it is impossible to give an average screening time, as it varies from case to case. The rule in this laboratory is that not less than five minutes should be spent screening the most innocent appearing smear. The normal cyclic pattern of the gynecological smear offers some reassurance but occasionally, also, offers surprises. Such reassuring patterns are not present in smears from other sources and warrant painstaking examination of the whole slide. This is particularly important in laboratories where these negative diagnostic smears are reported by the cytotechnologist.

EMMERICH von HAAM, Columbus, Ohio, U. S. A.:

I agree with Maria del Campo on the screening time for smears of the female genital tract. Smears from the respiratory tract, gastrointestinal tract and urinary tract take considerably longer. For the urinary tract and the large bowel we recommend the Millipore technique which requires that much larger specimens be screened and much smaller cells be studied. It takes from one-half to one hour to completely screen two Millipore filtered specimens from a colonic lavage or urine sediment.

PATRICIA KELLY, San Francisco, California, U. S. A.:

I believe the amount of time given to screening a smear should be the amount of time necessary to adequately examine the cells. Birgitta Fredrikson stated that this time varies from case to case, and I am in complete accord with her views. Emphasizing time rather than accuracy may have two unfortunate results. First, it may set a false goal for screeners since a cytotechnologist may feel that her work will be judged by quantity rather than quality. Secondly, a certain degree of accuracy may be lost. This is especially true when smears contain single malignant cells from endometrial adenocarcinoma, malignant oat cells from the respiratory tract, or the conditions cited by Birgitta Fredrikson. All the main speakers, I am sure, agree that accuracy is of paramount importance. However, I feel that by setting time averages —something that has never been done in hematology, bacteriology, etc.—accuracy will be sacrificed in an effort to maintain speed.

OLAF T. MESSELT, Oslo, Norway:

Maria Blanco de del Campo bases her judgment on an eight hour working day. In my opinion no cytotechnologist can sit fixed to a microscope for a period of eight hours and do satisfactory screening. In my laboratory even the most skilled cytotechnologist with up to ten years

experience is not allowed to do only screening, for more than a maximum of six hours per day. I agree with Birgitta Fredrikson that it is impossible to give an average screening time for vaginal smears and smears from breast secretion. This applied even more to the other specimens in question.

## CLOSING REMARKS

MARIO S. BLANCO de del CAMPO:

To Dr. Messelt:

An eight hour working day is interpreted as the time spent by a cytotechnologist in the laboratory; it is not an expression of uninterrupted work.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

# WHAT IS THE AVERAGE CAPACITY AS TO THE NUMBER OF CASES OF CYTOLOGICAL SMEARS PER DAY A CYTOTECHNOLOGIST CAN EITHER HANDLE, PROCESS AND SCREEN OR SCREEN ONLY?

BIRGITTA FREDRIKSON
Gothenburg, Sweden

The number of vaginal smears a cytotechnologist can screen per day is entirely dependent on what kind of material they represent and the aim of the investigation. At the cytology laboratory at the Cancer Hospital in Gotenburg we handle only slides from patients with gynecological disorders (i.e., cancer patients), patients with precancerous lesions (where the pathologist and gynecologist for some reason have recommended repeated cytological examinations), patients with clinically suspicious lesions or symptoms, radiologically treated follow-up patients, etc.

We have found that 20 slides is the maximum amount the cytotechnologist can screen per day. In material like ours where a great part of the smears contain atypical cells and where we try to give a detailed description and a differentiated diagnosis the cytotechnologist has to spend quite a long time on each smear. If he is forced to screen more slides, his attention will flag and he might easily overlook important cellular changes. When performing mass screening on symptom-free women, the capacity will of course increase.

COLETTE PASCAL, NICOLE DENAVIT
AND LUCIENNE TRONCY
Lyon, Rhône, France

It is evident that the number of smears a cytotechnologist can handle depends on whether the work consists of only screening slides or also of doing the technical procedures of preparing them. In the laboratory of cytology at the Institut Pasteur of Lyon, we think it is more advantageous if the cytotechnologist does not spend all his time at the microscope but also has the responsibility of preparing the slides to be screened, registering the cases and staining the incoming material. It is beneficial for the laboratory if the cytotechnologist can take care of the classification of the cases, establish a better relation with the clinical department and also be able to classify the material which is to be used for future publications of the laboratory.

We also believe that it is advantageous for the technician to stain the slides which he has to screen so as to see for himself the possible variability of colorations of the smears.

It also seems worthwhile in the cytological work to change one's occupation; in our opinion working continuously at the microscope produces fatigue which can be prejudicial with regard to the accuracy of the cytological diagnosis.

Taking the conditions into account it has been our experience that a technician can handle at least 25 or 30 slides a day including processing and screening. This number depends upon the difficulties of interpretation. These difficulties depend on various factors, and one may consider the time spent in screening related to the type of slides:

2 minutes for non-inflammatory smears,
5 minutes for inflammatory smears without dyskaryotic cells,
7 to 10 minutes for slides showing dyskaryotic cells
15 to 30 minutes for difficult smears (Class III).

The amount of 25 to 30 slides a day corresponds to the figure given by Cameron (1), even when the technician is responsible for the screening only.

Bibliography

1. Cameron, Ch. S.: First Internat. Cancer Cytology Congr., October, 1956.

## HOWARD L. RICHARDSON

Seattle, Washington, U.S.A.

Each laboratory will vary in its capacity to process and screen cases. Factors which contribute to this are organizational and will vary with the institution. Table I is a statistical analysis of the case load in the Strang Cytology Laboratory, New York City. Emerson Day, Director of the Strang Clinic, collaborated in this investigation in order to determine the future requirements of the Cytology Laboratory. In this operation training of cytotechnicians, teaching, diagnositic hospital work, and research were all performed. The average case load per technician was approximately 10 a day, or an average of 20 to 30 slides per day.

In comparison, Table II is a statistical analysis of the case load in a Cytology Laboratory in Seattle. The basic organization is the same as the Strang Cytology Laboratory except this laboratory is not connected with any institution. Teaching is not a factor. However, in the hospital diagnostic cytology and research are an integrated service. In this type of operation, it is calculated that the trained cytotechnician can handle approximately 24 to 30 cases per day, or 48 to 60 slides per day. Over and above this amount, fatigue and mental strain produce a consequent loss of accuracy.

## EDMUND SCHÜLLER AND MARGARETE PEUTSCH

Vienna, Austria

The average number of cytological smears which an experienced cytotechnologist is able to handle, process and screen, seems to lie between 50 and 100 per day.

He cannot screen many more because microscopic work depends on the efficiency of the eyes, which is limited.

Table I

Case Load
Strang Cytology Laboratory, New York

| Year | 1948 | 1949 | 1950 | 1951 | 1952 | 1953 |
|---|---|---|---|---|---|---|
| Pathologists | 1 | 1 | 1 | 1 | 2 | 2 |
| Resident pathologists | | | | | | 2 |
| Cytotechnicians | 2 | 2 | 5 | 6 | 8 | 8 |
| Clinical physicians | 3 | 5 | 4 | 3 | 4 | 4 |
| Secretary typists | | | | | 1 | 2 |
| Total case load | 7,801 | 10,505 | 13,016 | 14,043 | 17,744 | 20,182 |
| Case load for one technician per year | 2,900 | 5,252 | 2,603 | 2,340 | 2,218 | 2,522 |
| Case load per week (52 weeks) (per technician) | 56 | 101 | 50 | 45 | 43 | 49 |
| Case load per day (260 work days) (per technician) | 11 | 20 | 10 | 9 | 9 | 10 |

Table II

Case Load
Cytology Laboratory, Seattle

| Year | 1956 | 1957 | 1958 | 1959 |
|---|---|---|---|---|
| Pathologists | 1 | 1 | 1 | 2 |
| Resident pathologists | - | - | - | - |
| Cytotechnicians | 1 | 1 | 2 | 3 |
| Clinical physicians | - | - | - | - |
| Secretary typists | 1 | 2 | 3 | 3 |
| Total case load | 3,185 | 6,099 | 10,829 | 14,500 |
| Case load for one technician per year | 3,185 | 6,099 | 5,414 | 4,850 |
| Case load per week (52 weeks) (per technician) | 61 | 117 | 104 | 93 |
| Case load per day (260 work days) (per technician) | 12 | 24 | 21 | 19 |

## DISCUSSION

LUDWIG VON BERTALANFFY, Topeka, Kansas, U. S. A.
and
FELIX D. BERTALANFFY, Winnipeg, Manitoba, Canada:

Using the acridine orange fluorescence method introduced by the authors (1,2), the screening time may be reduced and the daily output of cytologic evaluations increased. The fluorescence technique according to Bertalanffy et al., requires six minutes for preparation of an individual specimen; if smears are processed in batches, more time may be saved. As with any technique, the required time for reading of a smear varies greatly in the individual cases. However, three minutes screening time per day is a fair average.

Fluorescence microscopy may be used either 1) as a prescreening procedure to rapidly screen out normal samples, and the suspicious samples, if preferred, may be processed according to any conventional technique or 2) for a final diagnosis.

The fluorescence method with its rapid procedure and easy recognition of malignant cells will be of advantage especially in the following circumstances:

1. In screening programs. For example, the fluorescence method was adopted at Walter Reed Army Hospital (3) as the routine method of exfoliative cytology. This group reports that the hourly output of screened slides was doubled.
2. In the gynecologist's office (4). The fluorescence method makes possible cytologic evaluation after short cytologic training. Rapid diagnoses (within 10 to 20 minutes) are also possible while the patient is waiting.
3. In the small hospital with limited facilities. Screening out of normal samples may be done by personnel with relatively little training, and only one cytologist is required to check the flourescence-suspicious samples and make the final diagnosis.

Bibliography


1. Bertalanffy, L. Von, Masin, M. and Masin, F.: Cancer 11:873, 1958.
2. Bertalanffy, L. Von and Bertalanffy, F. D.: Annals New York Acad. Sci., 1960.
3. Dart, L. H., Jr. and Turner, Th. R.: Lab. Invest. 8:1513, 1959.
4. Sussman, W.: Obst. & Gyn. 13:273, 1959.


MARIA S. BLANCO de del CAMPO, Palo Alto, California, U.S.A.:

I consider the maximum number of smears suggested by Birgitta Fredrikson as low even under the circumstances described in their laboratory, and I agree with Pascal, Denavit and Troncy in that the time spent screening is in direct relation to the quantity of the smear. However, when the duties of the cytotechnologist are limited to screening only, the average capacity as to number of cases (accepting as standard procedure two smears per patient—either vaginal and cervical or two from the same source) ranges from 30 to 40 cases. Thus, in this respect I agree with Richardson's estimates. The only time in which a cytotechnologist should be allowed to screen as many as 100 smears per day, as pointed out by Schüller and Peutsch, is when the laboratory has the following staff distribution:

a. Screener cytotechnologist—responsible for segregating entirely negative smears from those showing atypia, no matter how mild or infrequent, and negative smears from patients with histories of bleeding or other gynecological problems.
b. "Checker" cytotechnologist—responsible for the segregation of mild atypia from marked atypia.
c. Chief cytotechnologist—responsible for a more thorough study of atypical cases.
d. Pathologist—responsible for final decisions on cases with atypical features and/or atypical medical histories.

RUTH M. GRAHAM, Buffalo, New York, U. S. A.:

I agree with Birgitta Fredrikson that the number of slides that a technician can screen in one day is entirely dependent on the type of cytologic material examined. If the material sent to the laboratory is for routine screening purposes only, the technician can easily handle 40-50 slides a day. However, if the material is more difficult, including many problem cases, the number of slides screened per day will be much less. I have had the opportunity to compare the efficiency of technicians with both types of material. At the Vincent Memorial Laboratory of Boston, the majority of the slides were for routine screening purposes only. There, technicians were able to screen 50 slides a day with no difficulty. At the Roswell Park Memorial Institute of Buffalo, the material is very different. Any patient who is referred to the Institute must either have a definite diagnosis of cancer or that diagnosis must be seriously entertained. Our material has really been "screened" once before we see it. This type of cytologic material is very similar to that seen by Birgitta Fredrikson at the Jubilee Clinic of the Sahlgrenska Sjukhuset. Technicians seeing this kind of material screen 20 slides a day or just half the amount of a routine laboratory.

ELIZABETH H. GRAY, Ottawa, Ontario, Canada:

We must disagree with the statement of Schüller and Peutch, that an experienced cytotechnologist can handle, process and screen 50 to 100 slides a day. In a time study in our laboratory using the Papanicolaou staining technique, it was found that an experienced staining technician required a little over five hours to do the necessary book work, card indexing, staining, mounting and labelling of 100 gynecological smears. When non-gynecological material had to be processed another two minutes a slide were required. In a busy laboratory we believe it is not economical to use trained screeners for this work. They should, however, be thoroughly versed in staining procedures to be available as substitutes and to help out.

The statistical material furnished by Richardson is of particular interest. Our own screening load for 1959, with a working year of 262 days, averaged 42 slides per day, per technician. There is no allowance for loss of man hours through illness and vacations, or for the seasonal fluctuations in the daily number of specimens. This, in our laboratory, is a very real problem necessitating the screening of 80 to 100 slides per day per cytotechnologist in some of the winter months in order to maintain our 24 hour reporting service. This we understand is a universal problem and particularly serious where screening staffs are small and pathologist participation nominal. It is proving a serious deterrent to recruiting technicians into this field.

For these reasons we believe the policy of the Strang Cytology Laboratory of New York City of employing a screening technician for approximately every 20-30 slides per day to be the most desirable schedule presented. The intangibles are thus taken care of and a higher degree of accuracy and efficiency assured.

EMMERICH von HAAM, Columbus, Ohio, U. S. A.:

In our survey work where we deal mostly with non-flammatory smears or smears without evidence of severe dyskaryosis, our cytotechnologists are expected to handle 20 to 30 cases per day which amounts to between 30 and 50 slides, since not all cases are represented by two slides. This agrees with the figures of Richardson. In cases where a quantitative evaluation of the slide is required and a differential count of cells has to be done, up to one-half to one hour is needed for a single specimen.

IRENA KOPROWSKA, PRIMA BASKELIS and A. DARNELL CORSEY, Philadelphia, Pennsylvania, U. S. A.:

Presentations by Birgitta Fredrikson, by C. Pascal, et al., by Richardson, and by Schüller, et al., illustrate typical examples of a common experience in cytology laboratories. There seems to be a consensus of opinion that the number of gynecologic smears screened daily depends upon:

1. Whether population of screened women represents asymptomatic patients or problem cases with definite pathology, and
2. the organization and scope of activities of the cytology laboratory.

Interruptions in screening resulting from teaching obligations tend to decrease the average daily load of smears examined by a cytotechnologist. The screening capacity is also affected by the demand upon cytotechnologists to handle and process smears. As a matter of fact, the latter is considered beneficial in many cytology laboratories as part of the daily or weekly alternating routine. It seems, from figures presented by above authors, that in teaching institutions the expected average number of gynecologic smears screened by a cytotechnologist is 30 if the patients are asymptomatic, and 20 if one deals with patients from gynecologic clinics.

In non-teaching institutions, the output may be expected to be at least twice that much. In some instances, an experienced cytotechnologist apparently can process and screen 100 smears in the same day.

The experience presented by the above authors coincides with our own evaluation of the average screening capacity exhibited by cytotechnologists known to us. Although we are aware of a wide range of differences among individuals, the average screening output of a cytotechnologist, subjected to interruptions in screening by students in our laboratory, is 25 to 35 gynecologic slides in half a day. The remaining part of the day is usually devoted to non-screening activities. An experienced sputum screener screens, on the average, 60 to 80 slides daily. The most experienced sputum screener in our laboratory likes to screen all day on alternating weeks and spend full time handling and processing smears during remaining periods of time.

OLAF T. MESSELT, Oslo, Norway:

These questions are, according to my opinion, impossible to answer, as the answers will depend on a lot of circumstances varying from one laboratory to the other, for instance the organization of the laboratory and the complexity of the specimens sent to the laboratory.

JOSE R. DEL SOL, Madrid, Spain:

In our laboratory the cytotechnologists alternate handling and staining with screening. Our experience shows us that in a well-organized laboratory the number of cases that a cytotechnologist can process and stain is enormous in comparison to the amount that can be screened.

We believe that the maximum number of slides that a senior cytotechnologist can properly read in a normal work day is around 35-40. If the number of slides interpreted a day reaches 50 the proportion of errors increase significantly. We prefer a lower rate of screening in order to keep the relative proportion of errors as low as possible.

DOROTHY B. WELLER, Providence, Rhode Island, U. S. A.:

There is no doubt that an estimate of average work capacity depends on a variety of factors. To those mentioned by Birgitta Fredrikson and by Richardson, I would add minor considerations such as the length of the work day, variations in individual screening speed, and the amount of material on a slide.

In a mass screening of asymptomatic women, our cytotechnologists read 40-60 slides (an average of 24 cases) in 6-1/2 hours daily time at the microscope, with interruptions for lectures, discussions, teaching and study. No processing of slides is done by the screeners, although they have had this training and experience in the past. Frequent short breaks away from the microscope are considered necessary to avoid fatigue and to maintain diagnostic accuracy.

The estimates suggested by Pascal, Denavit and Troncy, concerning the relation of screening time to the type of slide, correspond to our experience. We receive many annual negative repeat cases which are read quickly, but our screeners also read all other cases, including suspicious and positive smears, for which they report a differential diagnosis to the pathologist and which often require a discussion, adding to the time spent on each case.

## CLOSING REMARKS

PIERRE HAOUR, C. PASCAL, N. DENAVIT and L. TRONCY:

From the above discussion it appears that 30 to 60 slides a day is considered within the average range that can be screened by a well trained cytotechnologist.

As is stressed by some of the discussants, this will depend upon the type of material sent to the laboratory. We agree that the material that has already been preselected by the gynecologist with the aid of the colposcope will require much more time to examine.

We have not had any personal experience with fluorescence microscopy, but after reading Bertalanffy's interesting report it would seem worthwhile to apply this technique to routine screening.

EDMUND SCHÜLLER and MARGARETE PEUTSCH:

The amount of work the screener may accomplish depends entirely upon his experience and training. We spoke merely about the conditions in gynecological cytology. If undisturbed working conditions are provided, we believe that an experienced cytotechnologist can handle 100 cases daily. In our laboratory our senior cytotechnologists are frequently required to handle such a work load due to the great amount of specimens we have to process and evaluate. Certainly we also have quite a few co-workers who are less experienced and therefore can handle only a smaller load properly.

# SHOULD THE CYTOTECHNOLOGIST BE ORGANIZED IN HIS OWN ORGANIZATION?

COLETTE PASCAL, NICOLE DENAVIT AND LUCIENNE TRONCY

Lyon, Rhône, France

We believe that the cytotechnician should be organized in his own organization. The type of responsibility of cytotechnicians varies in each laboratory: sometimes the technicians are only asked to prepare the slides and to do the first screening, sometimes they also have to sign out at least the negative cases, the cytologist handling the inconclusive or positive cases. At the risk of appearing pretentious we think that whatever the degree of the technicians' responsibilities, their role in a laboratory of cytology appears greater than in other specialties. As a matter of fact, the main advantage of cytology for cancer detection consists in its possibility as a mass screening procedure or at least as an extensive routine technique. We do not believe that this can be realized without the aid of well-trained cytotechnicians. The experience and speed of the cytotechnician increases with the number of years he has spent in cytology. To summarize, the reasons why we think that cytotechnicians should be organized in their own organization are the following ones:

a. Cytology is a specialized occupation differing from other branches of instruction in which technicians are usually trained.
b. Cytotechnicians associated with laboratories of cytology from their beginning might wish to be grouped together because of their experience.
c. This organization of technicians would have not only the advantages of bringing recognition to the specialty and protecting their interests, but also in facilitating the ties and exchanges between members in different towns and countries. We even think that it would be profitable for the cytologists to exchange, for a certain period, their cytotechnicians in order to become acquainted with different methods of work.

We think that this organization of technicians should be under the supervision of the respective National Societies of Cytology. The cytotechnicians could participate in meetings and also a few pages of cytological publications might be devoted to them. For the last year in France, thanks to the efforts of de Brux, a special training program in cytology has been instituted. However, the cytotechnical profession has not yet been differentiated in France from other laboratory staffs, as we believe it should be.

EDMUND SCHÜLLER AND MARGARETE PEUTSCH

Vienna, Austria

An organization of cytotechnologists is desirable, provided that the membership is able to present evidence of completed training.

Open permanent positions should only be filled by members of this organization. In this way one could prevent putting incompetent people into available positions. On the other hand, the organized cytotechnologists could exert some influence on the increase in their salaries.

## DISCUSSION

MARIA S. BLANCO de del CAMPO, Palo Alto, California, U. S. A.:

The reasons offered by the European authors for the organization of cytotechnologists within our own organizations are wise. However, in the United States of America, two national organizations already exist, namely the Inter-Society Cytology Council and the American Society of Clinical Pathologists, of which American cytotechnologists may be part, rather than attempting to create a third group. The ISCC continues furnishing cytotechnologists with responsibilities within the organization, and the ASCP-MT, in including our specialty in their society, protects and strives toward the enhancement of the group. To correct some of the obvious difficulties facing cytotechnologists unable to attend annual meetings or actively participate in either of these two organizations, regional or local chapters could be formed. They could select a delegate to represent their interests at meetings of the Executive Committee of the larger groups as well as at annual meetings.

RUTH GIBBERT, Munich, Germany:

It is also our opinion that the cytotechnologist should have his own organization. We also agree with the reasons for this as given by Pascal, Denavit and Troncy. Since cytology is really a very specialized field, the organization would help to make cytology a recognized specialty and, on the other hand, would enable the members of various countries and cities to exchange experiences. We also deem it advantageous if the cytologists, with the help of the organization, would be given the opportunity of exchanging their cytotechnologists. The cytotechnologists would become acquainted with various working methods and their otherwise somewhat one-sided activity would be enlivened by new stimuli.

Cytotechnologists would also welcome the opportunity of participating in meetings and publishing short cytological papers.

All cytotechnologists, in my opinion, should automatically belong to this organization. In other words, the membership should be mandatory. The annual fees would be kept low, since it would be an organization pursuing common interests, as a union.

In order to become a member of this organization, completed training should be a prerequisite. We agree with Schüller and Margarete Peutsch that Civil Service positions should be filled only with members of this organization.

It is obvious that the organized cytotechnologist would have a greater influence on the increase of salaries than would be possible for the individual cytotechnologist.

IRENA KOPROWSKA, Philadelphia, Pennsylvania, U. S. A.:

Among objectives listed in favor of setting up such organizations are: the need for achieving a better recognition of the field of cytotechnology and its responsibilities, and a desire of cytotechnologists to share experience and exchange ideas with individuals of similar interest. A hope was expressed that an improvement in salary scales may be expected for members of such organizations, especially if admission were restricted to qualified cytotechnologists. Cytotechnologists in the United States already may belong to the Inter-Society Cytology Council as Associate Members (non-voting), and it is possible that various regional groups of cytotechnologists are already organized without a general knowledge concerning their existence and activities.

Last year, the Philadelphia Society for Cytologic Study was organized by local cytotechnologists in Philadelphia. This group holds monthly meetings in different medical centers, and a cytologist speaker is often invited to discuss special problems. In the fall of 1959, the Philadelphia Society for Cytologic Study succeeded in organizing their own all-day symposium, which was very well attended and had an informative program. Presently, preparations are already under way for the second symposium scheduled for the fall of 1960. These activities appear to be beneficial and gratifying to participating cytotechnologists and, under proper leadership, probably will continue to retain respect and co-operation of local physicians. It is probable that the existence of such a specialized organization among medical technologists may help to stimulate interest in Exfoliative Cytology among other laboratory technicians.

F. A. LANGLEY, Manchester, England, U. K.:

In Great Britain I think it would be better that cytotechnologists should be organized with other laboratory technicians in the Institute of Medical Laboratory Technology. The reasons for this have been given by Taylor elsewhere in this Symposium. Nevertheless, it would be well if they had some loose association with each other and with pathologists in order to exchange ideas and learn what is going on elsewhere. To some extent this can be achieved by cytotechnologists visiting and working in other laboratories for short periods.

PASCO R. DEL VECCHIO, Bethesda, Maryland, U. S. A.:

The two papers submitted for discussion bring to light some interesting points. I agree that cytology is a specialized occupation which differs from other branches of laboratory work. The cytotechnologist many times must assume the responsibility for taking, preparing and reporting the results of the specimens obtained. As mentioned by Pascal and co-workers, one way to assure more competent people is for cytotechnologists to be organized as an integral part of an organization, such as the Inter-Society Cytology Council. Being part of an organization of this type, with a cytotechnologist as a member of the governing body of the Society, would insure the interests of the cytotechnologists and best further the whole field of Cytology.

I feel that by forming their own independent organization, the cytotechnologists may jeopardize their interests and the quality of cytodiagnosis in general.

## CLOSING REMARKS

PIERRE HAOUR, C. PASCAL, N. DENAVIT and L. TRONCY:

The interest in setting up organization of cytotechnologists as part of a Society for Cytology is accepted by the authors. We wish that this question be raised at the International Congress of Exfoliative Cytology in Vienna, Austria in 1961.

EDMUND SCHÜLLER and MARGARETE PEUTSCH:

Our country, Austria, is too small to reasonably found a separate organization for cytotechnologists. This is the reason why, in this country the few persons who would organize such a group attempt to become associated with already larger existing organizations.

# SHOULD A CERTAIN PERIOD OF TRAINING THE CYTOTECHNOLOGIST BE DEVOTED TO WORK IN THE PATIENT'S CARE? IF SO, WHICH FIELDS AND WHICH ACTIVITIES SHOULD BE EMPHASIZED?

BIRGITTA FREDRIKSON
Gothenburg, Sweden

I do not believe it necessary for the training period to include care of the patient, since there will always be persons from the staff available on the wards. On the other hand, the cytotechnologist will need instructions from a gynecologist on how to handle the instruments needed for taking smears from the cervix and the cervical canal. Instructions concerning the appearance of the normal, and to a certain extent the abnormal, cervix are of importance for correctly taken smears. It might also be of value to become acquainted with the changes in the cervix caused by radium treatment.

RUTH GIBBERT
Munich, Germany

Some knowledge of diseases of the female reproductive tract and some insight into the working conditions of the outpatient Clinic or the doctor's office belongs indispensably to the training of a cytotechnologist. He will do his subsequent work in the laboratory with much more interest and understanding if he knows what has gone on previous to the smear's being taken and if he has some idea of the clinical aspect of the cytological smear.

The training period in an office or outpatient clinic will always have to be a short one. However, the trainee should spend sometime at first assisting the physician in the examination of the patient. He may watch the doctor doing colposcopy and also study colpophotographs. The physician may then show him how to prepare cervical smears with various spatula for early cancer detection and how to take smears with the cotton swab from the lateral vaginal wall for hormonal evaluations. Subsequently he may take smears for himself, fix them and afterwards look at these slides in the laboratory. This will give him some idea of how so many mistakes in smear preparation occur and also of how difficult it is to screen ill-prepared smears. Furthermore, while in the outpatient clinics or in the doctor's office he will hear the words "clinical diagnosis: erosion," for example, and he will write down all these technical terms new to him and fill in some explanatory notes later for further reference. For instance: Leukoplakia: thickening of the squamous epithelium; colposcopically: white spot on the cervix; Colpitis: vaginal inflammation, etc. He can learn these terms and continue to gather information about them. Moreover he should learn something about the treatment of various diseases with which he is working. In the event that a patient is admitted to the hospital from the outpatient Clinic, he should then know why the hospitalization was necessary and what the further treatment would be. He should acquaint himself with the treatment of early carcinoma. The best system would be if the trainee, himself, could work for some time on the floors in order to deepen the knowledge acquired in the outpatient Department and in order to be able to further follow-up the cases.

In the final analysis, it all depends upon the initiative of the individual trainee. A clinic and a hospital are a living organism where one may watch and learn beyond the required assignments. The trainee has the constant possibility of questioning the doctor in charge on those things which are uncertain to him. A wise man, once asked how he had acquired his universal knowledge, is said to have answered, "I was never ashamed to ask questions."

## DISCUSSION

MARIA S. BLANCO de del CAMPO, Palo Alto, California, U. S. A.:

If the term "patient's care" as used here means "nursing care," I agree with Birgitta Frederikson. It is not necessary to devote any period in the training of cytotechnologists to care of the patient. On the other hand, if the term signifies a bedside manner in the collection of specimens or assisting the physician during the procedure, I agree with Ruth Gibbert. Furthermore, it may be desirable under certain circumstances, viz. hospital lab-

oratories with a small work load (for example about 10 cases a day) or laboratories with a large diversified staff, that the collection of specimens be one of the routine duties of the cytotechnologist. In the United States of America the law forbids a non-medical person from collecting specimens which require special instrumentation, with few exceptions, such as gastric lavages.

The fields and activities requiring special attention as listed by Ruth Gibbert, although good, do not serve a practical purpose in the training of cytotechnologists in this country. It seems wise, however, to make the trainee aware of the need to win the patient's confidence and to apply the knowledge in bedside care in the collection of vaginal and gastrointestinal material, drawing of venous blood for peripheral blood cytology and use of new instruments for sputa collection, to name a few areas where he may profit by such knowledge.

HANNAH PETERS, Copenhagen, Denmark:

I cannot agree with Ruth Gibbert that the training of a cytotechnologist should include the care of the patient. It will be difficult to define what shall and what shall not be included in a training program, but we must not lose sight of the ultimate aim: to develop capable, experienced technicians in cytology. Birgitta Fredrikson's suggestion that a cytotechnologist learn to insert a speculum and take smears from the cervical canal will not be favored in all quarters. I believe it is a good rule to say that whenever instrumentation is necessary a medical doctor should be present. I would be hesitant to let a technician routinely take endocervical or endometrial smears.

CHARLOTTE M. STREET, New York, New York, U. S. A.:

I agree with Birgitta Fredrikson concerning this question. Some knowledge of the diseases of the female genital tract and their treatment undoubtedly stimulates the cytotechnologist's interest in cytologic diagnosis, but it can be presented as part of the theoretical training. His time can be spent much more profitably in microscopic study than in the details of patient care.

## CLOSING REMARKS

BIRGITTA FREDRIKSON:

As in Sweden there is no law against the cytotechnologist taking the smears, they are taught to do so. As a rule the doctor takes the smears before examining the patient, but when repeated smears are wanted it might be more convenient and time saving for both patient and doctor if the cytotechnologist is able to take it. It is sometimes useful if she can take smears on the wards also.

RUTH GIBBERT:

The discussion showed differences of opinion on two points.

The training program on the wards of the hospital would certainly be good. However, it seems that such an extension of the program would make the total time of the teaching period too long.

Concerning the preparation of the specimens from the patient: we believe that this is not within the province of the cytotechnologist.

# WHAT IS THE OPTIMUM LENGTH OF TIME FOR TRAINING?

JEAN A. de BRUX
Paris, France

The answer to this question is obviously variable, and depends on: a) the intelligence, background, diligence, the desire to learn and the ambition of the beginning technician, b) the quality of the instruction (knowledge, teaching talent and patience of the instructor) and c) the number and the varieties of smears screened.

If the practical program (which we have suggested in a preceding topic) is followed, at the end of six months an average cytotechnician can, as a rule, have acquired enough ideas in hormonal and cancer cytology to render himself useful in the routine work of a laboratory. His work should be verified.

At the end of a year, if he has continued his efforts and if he readily accepts advice, his training may be considered completed.

## DISCUSSION

J. ERNEST AYRE, Miami, Florida, U. S. A.:

In general we recommend a period of six to twelve months full time study and training to become well qualified in general cytology diagnosis. There are exceptions to the rule, however, and some exceptional students can qualify in lesser periods of time, e.g., three to four months. In all cases this should depend on the ability of the student to pass oral and practical examination after study in laboratories where there is an active flow of gynecological and non-gynecological specimens.

BIRGITTA FREDRIKSON, Gothenburg, Sweden:

I agree with the speakers that the minimum time for education is dependent on a variety of personal factors, but that one year of training ought to be a minimum.

ELIZABETH H. GRAY, Ottawa, Ontario, Canada:

After more than ten years experience as a cytotechnologist, I can truthfully say I am still learning and do not feel that training in this field can ever be considered "completed." I do, however, agree with both above authors that the initial training should not be less than a year. This should be divided between four to six months formal study, followed by the balance of the year in a supervised screening laboratory under a pathologist who is qualified in cytology.

PATRICIA KELLY, San Francisco, California, U. S. A.:

The length of time and the type of instruction described by the above authors seems most advantageous and students trained in this manner should become very well qualified cytotechnologists. It is logical that the longer one spends on the study of a subject, the greater his understanding will be of the topic. Nevertheless, I feel that some consideration must be given to the amount of responsibility the cytotechnologist will assume after completion of his formal training. If he is to be a part of a laboratory where he will discuss smears with more advanced cytotechnologists and where his work will be reviewed and verified by a cytologist, I believe four to six months training is adequate. However, the study of cytology should not cease after completion of his formal training. The graduate cytotechnologist should endeavor to extend his knowledge through the patient and detailed study of cells and through the study and discussion of pertinent books and journals.

I would advocate a one or two week workshop given yearly for experienced cytotechnologists as a means of providing continuation of study without causing too extensive a break in the routine schedule of the various laboratories. The program might be sponsored by teaching laboratories and given regionally or locally for a limited number of technicians. Smears, from the participating laboratories, presenting unusual problems or contributing new information in cytology could be mailed in advance to the sponsoring laboratory for processing. These smears could then be examined and discussed by the visiting cytotechnologists. Lectures and demonstrations of latest techniques would, of course, play an important part in such a program.

IRENA KOPROWSKA, Philadelphia, Pennsylvania, U. S. A.:

The broadminded approach of de Brux to this question, with his consideration of variables inherent in the quality of students, instructors and teaching material, renders any ensuing detailed discussion rather superfluous. The opinion of del Sol that six-month courses are inadequate unless the trainees are college graduates is supported by our experience. In our laboratory, we offer six- and twelve-month training programs for college graduates.

Each of these programs comprises identical theoretical and practical instruction in handling and evaluation of different types of cytologic material. Six-month programs are usually taken by tuition-paying students. A twelve-month course is made available to students accepted to the laboratory to acquire training on the job, who, while studying, are considered to be employees of the laboratory. They are obliged to perform, in between periods of study, definite service chores such as staining, filing and coverslipping slides, doing clerical work, etc. Courses of shorter duration than six months, in our opinion, tend to result in less adequate training. Training programs longer than twelve months can hardly be recommended for practical reasons. Graduate students of six- and twelve-month courses still need one to two years of additional experience and a certain amount of guidance before they become truly competent screeners.

PIERRE HAOUR, C. PASCAL, N. DENAVIT and L. TRONCY, Lyon, Rhône, France:

We agree with de Brux that many factors may determine the length of time for the training of cytotechnicians. The length of time for theoretical training will be quite variable, according to the nature of the program of studies. As it is developed elsewhere in this Symposium, it appears that a six-month training can be considered the minimum requirement. In order to fully learn to screen slides correctly, a period of six-months will not be sufficient. The knowledge of normal patterns of cytology for hormonal diagnosis and of cell counts may be acquired more rapidly, but for cancer screening the cytologist himself must check the slides screened by the student cytotechnician much longer than six-months after the onset of training.

JOSÉ R. DEL SOL, Madrid, Spain:

Our experience is that a capable cytotechnologist needs one year of training to be able to work at a maximum speed and sureness.

We divide the training into three, four-month periods. During the first period we emphasize the theoretical work (described in another section of this Symposium). The second and third periods are dedicated almost exclusively to practical training.

Due to the need for trained cytotechnologists, we tried giving an abbreviated course of six months duration. The results were not completely favorable, except in those cases where the individual had a high degree of previous education (university graduates with a knowledge of foreign languages).

We have come to the conclusion that periods of training longer than one year are unnecessary, because in this length of time the fundamental points have been acquired; further knowledge is best obtained during the daily laboratory work rather than in a special training course.

CHARLOTTE M. STREET, New York, New York, U. S. A.:

Neither of the above authors has specified whether he refers to training in gynecological cytology only or also to the application of the method to all systems. I agree, however, with de Brux that the length of time depends upon the qualifications of the trainee and the quality of instruction given.

On the average, I would say that one year is the optimum length of time for training to produce a cytotechnologist who will be an accurate and reliable screener in all phases of exfoliative cytology. This would include six months of intensive training and six months of practical experience in screening, whether the latter be in the same institution where the formal training is given or in another cytology laboratory where his work can be checked by other more experienced screeners.

GUILLERMO TERZANO and ARTURO A. ARRIGHI, Buenos Aires, Argentina:

In agreement with de Brux we would like to emphasize the importance of the background and the previous training of the cytotechnologist, apart from the intelligence of the candidate. For those familiar with any kind of microscopical routine work such as examination of urine sediment, blood cytology, etc., it seems much easier to become acquainted with the exfoliated epithelial cells. In our laboratory as an average (with individual variations) a student spends some time (at least one month) in learning vaginal smears for endocrinological purposes, until he (or she) acquires satisfactory knowledge of the normal vaginal cells, percentages of each type of cell, curve of desquamation during the menstrual cycle, etc. After one month or so devoted to the study of normal cytology, he can commence the search for cancer cells in previously selected slides, starting with cases of squamous cell carcinomas, which show the most striking features and bizarre forms. We advise students to compare cells in smears with cells shown in the plates of textbooks, and also, to make drawings by themselves. Once he feels familiar with malignant cells (it takes one to two months), he can go over miscellaneous positive, suspicious and negative cases, with the clinical history attached; he is asked to write a report of the cases. When he "passes this test," as he usually does, he is invited to participate in the study of the interesting cases of the daily routine work. It gives the student the opportunity to discuss positive and suspicious cases and learn how to correlate cytological findings with the clinical history of the patient and the final histopathological diagnosis. If the student seriously continues his training for a longer period of time, in six months he can be considred qualified as a cytotechnologist who can carry on this kind of work independently. Provided that he is an honest and a capable worker, he will improve and advance by studying his own cases. For smears of other secretions and body fluids, it also depends mostly on the knowledge of the normal epithelial cells that appear in sputum, bronchial washings or aspirations, gastric specimens, urine, etc. We have little experience in teaching other than vaginal cytology smears, but we know by our experience that it is harder.

DOROTHY B. WELLER, Providence, R. I., U. S. A.:

I agree with de Brux. His clear, concise treatment of this question covered the subject completely.

## CLOSING REMARKS

JEAN A. de BRUX:

I fully agree with Elizabeth Gray, that the training of a cytotechnologist is never completed. Nor, for that matter, is that of any profession.

# SHOULD THE TECHNOLOGIST BE TRAINED IN ALL ASPECTS OF DIAGNOSTIC CYTOLOGY, I.E., SPUTUM, BRONCHIAL ASPIRATION, URINE, GASTRIC SECRETION, ETC.?

RUTH M. GRAHAM
Buffalo, New York, U.S.A.

It is an advantage if the technician can be trained in all aspects of diagnostic cytology. This is not always possible, since in many laboratories there is little material other than gynecologic.

Technicians can become more familiar with the morphology of certain cells if they are trained in all aspects of diagnostic cytology. Cells that are unusual in one type of specimen may be frequently encountered in a specimen from another source. There are numerous examples of this. Histiocytes with two or three nuclei are not common in smears from the female genital tract. In sputum they are a usual finding. Multinucleated squamous epithelial cells are seen infrequently in vaginal smears, yet in urine smears they are not at all unusual. If the student has seen smears from bronchial washings and has a working knowledge of the appearance of well preserved bronchial columnar cells, he will be able to recognize the well preserved endocervical cell with greater certainty.

The difficulty in diagnostic cytology is in accurately recognizing the many morphologic variations of the benign cells. The student can learn a great deal concerning the many alterations which occur in benign cells by studying sputum, gastric and esophageal washings, pleural and peritoneal fluids and urinary sediment. It is important that the student be exposed to these other types of specimens even though he may not expect to screen them in his daily work.

EMMERICH von HAAM
Columbus, Ohio, U.S.A.

My brief answer to this question is, "Yes," and my reasons for this answer are as follows:

Most of the cytotechnologists are now recruited from the ranks of medical technologists who are already trained in the cytology of blood and urine sediments. The rapid progress of cytology makes this method useful for the recognition of more and more disease processes, and to limit the training of cytotechnologists to vaginal smears alone could be compared to limiting the training of medical technologists in biochemistry to the determination of blood sugars because this is the most frequently requested procedure. In order not to forget what she has learned, it is of course necessary for her to continue to practice it, and she should stimulate her employer to examine other cytodiagnostic material in addition to vaginal smears. In the meantime she can maintain her acumen by examining positive and negative sample slides she will receive at the end of her training course, or she can prepare a slide collection of her own. Most of the books on clinical cytology include all aspects of diagnostic cytology, and I see no reason why cytotechnologists' training should be limited to vaginal cytology, even if this is the most widely used method at the moment. This should be left to the physician and can either be accomplished by the cytopathologist, if one is available, or by the referring physician. In the same way, we do not train cytotechnologists to critically judge the effect of therapy or recommend such therapy.

## DISCUSSION

RACHEL ACHENBACH, Salem, Massachusetts, U. S. A.:

I agree wholeheartedly that the cytotechnologist should take full advantage of the training we hope she has received, as suggested by Ruth Graham. It is also to her personal advantage if she maintains her proficiency in the examination of specimen types rarely encountered in her routine work, as von Haam suggests.

It is a sad and disturbing experience for a technician to change her job only to find that she has allowed her proficiency in certain areas to become atrophic. Her situation is no less unhappy if she should find that it was poorly developed in the first place.

J. ERNEST AYRE, Miami, Florida, U. S. A.:

It is most desirable for the average cytotechnician or cytologist-screener to be trained in all aspects

of cytotechnology. One exception to this would be in diagnostic and research cytology laboratories in departments of gynecology and obstetrics where cytology is exclusively limited to gynecological specimens.

ELIZABETH H. GRAY, Ottawa, Ontario, Canada:

Like von Haam, I think the only answer is "yes." His reasoning is unassailable. It is quite possible for the cytotechnologist to stimulate interest in cyto-diagnosis in addition to vaginal smears. From very meager beginnings (last year 20% of our work was non-gynecological) we show a considerable increase. Cytology's new strides will be in these fields.

IRENA KOPROWSKA, Philadelphia, Pennsylvania, U. S. A.:

I agree fully with the above authors that it is of definite advantage if the technician can be trained in all aspects of diagnostic cytology. A fully-trained cytotechnologist obviously has a greater familiarity with a variety of cellular material than one whose experience was limited to a single type of secretion. At the present stage of continued development of exoliative cytology, outside of the field of gynecologic cytology, one would hesitate to advise a prospective cytotechnology student to avail herself of an instruction limited to any one single type of cytologic material with exclusion of other aspects of diagnostic cytology. The time allotted to training in various fields of cytotechnology, however, may vary from one teaching center to another, depending upon its participation in the handling of different types of cytologic material.

HANNAH PETERS, Copenhagen, Denmark:

I agree with von Haam: training of cytotechnologists should be as complete as possible, and not be confined to the specialty of a particular laboratory.

HOWARD L. RICHARDSON, Seattle, Washington, U. S. A.:

Cytotechnicians should screen and recognize abnormal cells from multiple sites. However, in a cytology laboratory servicing practicioners of medicine the technician is fortunate to receive each month the following numbers of various cases: four sputa, one bronchial secretion, two urinary specimens, and only rarely a gastric specimen. In larger centers the specialty services readily supply the material and the accuracy of the method is now well established. Errors in diagnosis in large and small operations can be aleviated by the combined use of the smear and cell block techniques. In this way the cytotechnologist and pathologist are educated and the diagnostic results are increased by an additional 12%.

CHARLOTTE M. STREET, New York, New York, U. S. A.:

I agree with Ruth Graham and von Haam that technicians should be trained in all aspects of diagnostic cytology, and agree with the reasons which they have advanced. Furthermore, although a cytotechnologist's first position may be in a laboratory where only gynecological cytology is practiced, he may be called upon at any time to screen another type of smear, or he may take a subsequent position where he will be expected to screen all types of smears. If he has received sound diversified training in the beginning, he should be qualified after some review, to fill such a position.

GUILLERMO TERZANO, Buenos Aires, Argentina:

Ruth Graham and von Haam have such a great deal of experience with students that it is gratifying to read their statements. It is easy to agree and accept their interesting points of view. Though originally a gynecologist, I am interested in all aspects of diagnostic cytology and not exclusively in vaginal smears. It seems reasonable to believe that the careful study of smears from other sources contributes to the formation and education of a cytologist. It is not unusual for a beginner to interpret erroneously some abnormal cells and even to make "a correct diagnosis on wrong cells." I know it from my own experience. It happened the day I marked with dots a couple of clusters of histiocytes (which I proudly called Class IV epithelial cells) and overlooked a small group of actual cancer cells desquamated from a carcinoma of the stomach.

DOROTHY B. WELLER, Providence, Rhode Island, U.S.A.:

I am aware of the need for a complete training course in cytology, because our laboratory is limited to gynecological study. Some of our technicians have studied the theory of extravaginal cytology; however, since no specimens have been available for practical study, their knowledge and experience is quite inadequate for diagnostic work with other than vaginal and cervical smears.

On the other hand, I agree with von Haam that the technician will quickly forget, if she does not use and continue to practice what she has learned. Therefore, it would seem advisable for most small laboratories to train specifically for the work to be done, with the hope that, given an opportunity to work in another aspect in cytology, the technician who is well-trained in one, will quickly become familiar with all types of specimens.

## NO CLOSING REMARKS

# ALPHABETICAL LIST OF PARTICIPANTS OF PRESENT SYMPOSIA BY CORRESPONDENCE

1. RACHEL R. ACHENBACH, Danvers, Massachusetts, U.S.A. (2 Poplar St.)
2. MICHELYNE ANCLA, Paris, France (c/o Prof. Jean A. de Brux)
3. ARTURO ANGEL ARRHIGI, Buenos Aires, Argentina (St. Martin de Tours 2952)
4. MARGARET E. ATTWOOD, Birmingham, England, U.K. (Queen Elizabeth Hospital)
5. J. ERNEST AYRE, Miami, Florida, U.S.A. (1700 Biscayne Blvd.)
6. GENEVIEVE BADER, New York, New York, U.S.A. (Memorial Center for Cancer)
7. GUNTER F. BAHR, Washington, D.C., U.S.A. (Armed Forces Institute of Pathology)
8. FRIEDRICH BAJARDI, Graz, Austria (Brockmanngasse 14)
9. FELIX BERTALANFFY, Winnipeg, Manitoba, Canada (University of Manitoba)
10. LUDWIG von BERTALANFFY, Topeka, Kansas, U.S.A. (Menninger Foundation)
11. WERNER BICKENBACH, Munich, Germany (Maistrasse 11)
12. MARIA S. BLANCO de del CAMPO, San Juan, Puerto Rico (P.O. Box 665)
13. HANNS-WERNER BOSCHANN, West Berlin, Germany (Kaiserdamm 29)
14. JEAN A. de BRUX, Paris, France (11 Bis Rue Dufrenoy)
15. DIETRICH BUTTENBERG, Heidelberg, Germany (Univ.-Frauenklinik)
16. JADWIGA BYLINA, Gliwice, Poland (Instytut Onkologiiul. Czerwoney Armii 15)
17. ALICE DARGAN CLARK, Chicago, Illinois, U.S.A. (5841 S. Maryland Ave.)
18. NANCY DAWSON, Newcastle upon Tyne, England, U.K. (c/o Dr. Taylor)
19. EMERSON DAY, New York, New York, U.S.A. (Memorial Center for Cancer and Allied Diseases)
20. NICOLE DENAVIT, Lyon, Rhône, France (6 Place Chazette)
21. CARY M. DOUGHERTY, Baton Rouge, Louisiana, U.S.A. (707 N. Seventh St.)
22. JACQUELINE DUPRÉ-FROMENT, Paris, XVIIe France (6 Rue Anatole de la Forge)
23. GRACE R. DURFEE, New York, New York, U.S.A. (Memorial Center for Cancer)
24. JAMES HENRY FERGUSON, Miami Florida, U.S.A. (University of Miami)
25. CLARICE do AMARAL FERREIRA, Rio de Janeiro, Brazil (Emilio Berla, 46 Apto. 302)
26. ALVAN G. FORAKER, Jacksonville, Florida, U.S.A. (800 Miami Road)
27. BRIGITTA FREDRICKSON, Gothenberg, Sweden (Cytologiska Lab. Jubileum skliniken Sahlgrenska Sjukhuset)
28. MARCEL GAUDEFROY, Lille, Nord, France (94 Rue d'Isly)
29. RUTH GIBBERT, Munich, Germany (I. Universitaets-Frauenklinik)
30. RUTH M. GRAHAM, Buffalo, New York, U.S.A. (666 Elm St.)
31. ELIZABETH H. GRAY, Ottawa, Ontario, Canada (Ottawa Civic Hospital)
32. EMMERICH von HAAM, Columbus, Ohio, U.S.A. (1997 Cambridge Blvd.)
33. PIERRE HAOUR, Lyon, Rhône, France (43 Rue Duquesne)
34. CONSTANTIN HEROVICI, Paris, Villejuif, Seine, France (16 Bis, P.V. Couturier)

35. I. HLINIAK, Gliwice, Poland (Instytut Onkologii ul. Czerwoney Armii, 15)
36. SARA HODGES, Santurce, Puerto Rico (250 Parque St.)
37. B. CORNELIUS HOPMAN, Miami, Florida, U.S.A. (Jackson Memorial Hospital)
38. WARREN C. HUNTER, Portland, Oregon, U.S.A. (3181 S.W. Sam Jackson Rd.)
39. HANS HUSSLEIN, Vienna XVIII, Austria (Bastiengasse 36-38)
40. JEAN L. JOHNSON, Buffalo, New York, U.S.A. (666 Elm St.)
41. MARIA KAWECKA, Gliwice, Poland (Instytut Onkologii ul. Czerwoney Armii, 15)
42. PATRICIA KELLY, San Francisco, California, U.S.A. (476 Diamond St.)
43. OLLE KJELLGRAN, Gothenberg, Sweden (Sahlgrenska Sjukhuset)
44. IRENA KOPROWSKA, Philadelphia, Pennsylvania, U.S.A. (235 N. 15th St.)
45. WOLFGANG KORTE, Bonn, Germany (Poppelsdorfer Allee 51)
46. LEOPOLD G. KOSS, New York, New York, U.S.A. (444 East 68th St.)
47. HANNES KREMER, Vienna, Austria (Spitalgasse 23)
48. RAIMUND KRIMMENAU, Dresden, Germany (Frauenklinik der Med. Akade.)
49. ERNST H. KRÜGER, Halle a.d. Saale, Germany (Leninstrasse 16)
50. TED LACH, Buffalo, New York, U.S.A. (666 Elm St.)
51. WARREN R. LANG, Philadelphia, Pennsylvania, U.S.A. (1912 Walnut St.)
52. F.A. LANGLEY, Manchester, England, U.K. (St. Mary's Hospital for Women and Children)
53. MARTIN H. MATZ, Miami, Florida, U.S.A. (c/o Dr. James Henry Ferguson)
54. ALEXANDER MEISELS, México, D.F., México (Obrero Mundial 240-102)
55. OLAF T. MESSELT, Oslo, Norway (The Norwegian Radium Hospital)
56. EDWARD M. MILLER, JR., Columbus, Ohio, U.S.A. (2585 Shrewsbury Rd.)
57. LUIS MONTALVO-RUIZ, Madrid, Spain (Lagasca 7)
58. HERBERT E. NIEBURGS, New York, New York, U.S.A. (4 East 95th St.)
59. VIOLETTE M. NUOVO, Paris, France (5, Rue de Magdebourg)
60. OTAKAR NYKLÍČEK, Náchod, Czechoslovakia (Nemocnice)
61. COLETTE PASCAL, Lyon, Rhône, France (16, Rue Grenette)
62. HANNAH PETERS, Copenhagen, Denmark (Onsgaardsvej 10)
63. MARGARETE PEUTSCH, Vienna, Austria (II. Universitaet-Frauenklinik, Spitalgasse 23)
64. J. PAUL PUNDEL, Luxembourg, Luxembourg (23 Rue Albert ler)
65. ABRAHAM E. RAKOFF, Philadelphia, Pennsylvania, U.S.A. (269 South 19th St.)
66. HOWARD L. RICHARDSON, Seattle, Washington, U.S.A. (3049 Esplanade Drive)
67. GEORGE L. ROMBERG, White Plains, New York, U.S.A. (145 Maple Ave.)
68. EDMUND SCHÜLLER, Vienna, Austria (Spitalgasse 23)
69. HORST SMOLKA, Kiel, Germany (Hegervischstrasse 4)
70. JOSÉ R. del SOL, Madrid, Spain (Alberto Bosch 10)
71. YOSEUP S. SONG, Providence, Rhode Island, U.S.A. (593 Eddy St.)
72. HANS-JÜRGEN SOOST, Munich, Germany (Maistrasse 11)
73. MARVIN W. STAFFORD, Philadelphia, Pennsylvania, U.S.A. (3426 Conrad St.)

74. H. STARKIEWICH, Gliwice, Poland (Instytut Onkologii, Czerwoney Armii 15)

75. PETER STOLL, Heidelberg, Germany (Helmholtzstrasse 2)

76. CHARLOTTE M. STREET, New York, New York, U.S.A. (Cornell University Medical College)

77. CLAUDE W. TAYLOR, Birmingham, England, U.K. (Birmingham and Midland Hospital for Women)

78. GUILLERMO TERZANO, Buenos Aires, Argentina (St. Martin de Tours 2952)

79. LUCIENNE TRONCY, Lyon, Rhône, France (36, Rue Laennec)

80. PASCO R. del VECCHIO, Bethesda, Maryland, U.S.A. (Nat'l. Inst. of Health)

81. ERICA WACHTEL, London, England, U.K. (Hammersmith Hospital)

82. WOLFGANG WALZ, Heidenheim a.d. Brenz, Germany (Kreiskrankenhaus, Frauenklinik)

83. STANLEY WAY, Newcastle-Upon-Tyne, England, U.K. (Queen Elizabeth Hospital)

84. DOROTHY B. WELLER, Providence, Rhode Island, U.S.A. (593 Eddy St.)

85. GEORGE L. WIED, Chicago, Illinois, U.S.A. (5841 S. Maryland Ave.)

# SYMPOSIA UNDER CONSIDERATION

The following symposia have been suggested for consideration and are not listed in the order of preference of chronology. You are invited to inform the Editorial Office in which of the symposia you would be most interested, so that an order of preference may be tentatively arranged.

Symposium on Tadpole Shaped Squamoid Cells

Symposium on Cytological Studies in Amenorrhea

Symposium on Cytology of Ascitic Fluid

Symposium on Cytology of Malignant Tumors of Ovary and Tubes

Symposium on Extra-Genital Cytology of Metastatic Gynecological Lesions

Symposium on Phasemicroscopy and Other Special Microscopic Techniques

Symposium on Training of Exfoliative Cytologists

Symposium on Cytological Changes due to Microbiological Factors

Symposium on the Comparative Diagnostic Accuracy, Efficiency and Specificity of Techniques for Detection of Carcinoma

Symposium on Cytological Microphotography

Symposium on Cytological Terminology for Hormonal Evaluation

Symposium on Quantitative Cytochemistry of Exfoliated Cells

Symposium on Vaginal Cytology During Childhood

Symposium on Lung Cytology

Symposium on Cytology of Exudates

Symposium on Gastrointestinal Cytology

Symposium on Endocervical Adenocarcinoma

Symposium on Oral Cytology

Symposium on Cytology of Nipple Discharge

Symposium on Squamous Cell Cancer of the Cervix with Particular Emphasis on Micro-Invasive Lesions

Symposium on Incidence Statistics of Cervical Carcinoma

Symposium on Tumor Cells in the Peripheral Blood

Symposium on Terminology such as "Abnormal," Suspicious," "Doubtful," and Others

Symposium on The Menopause

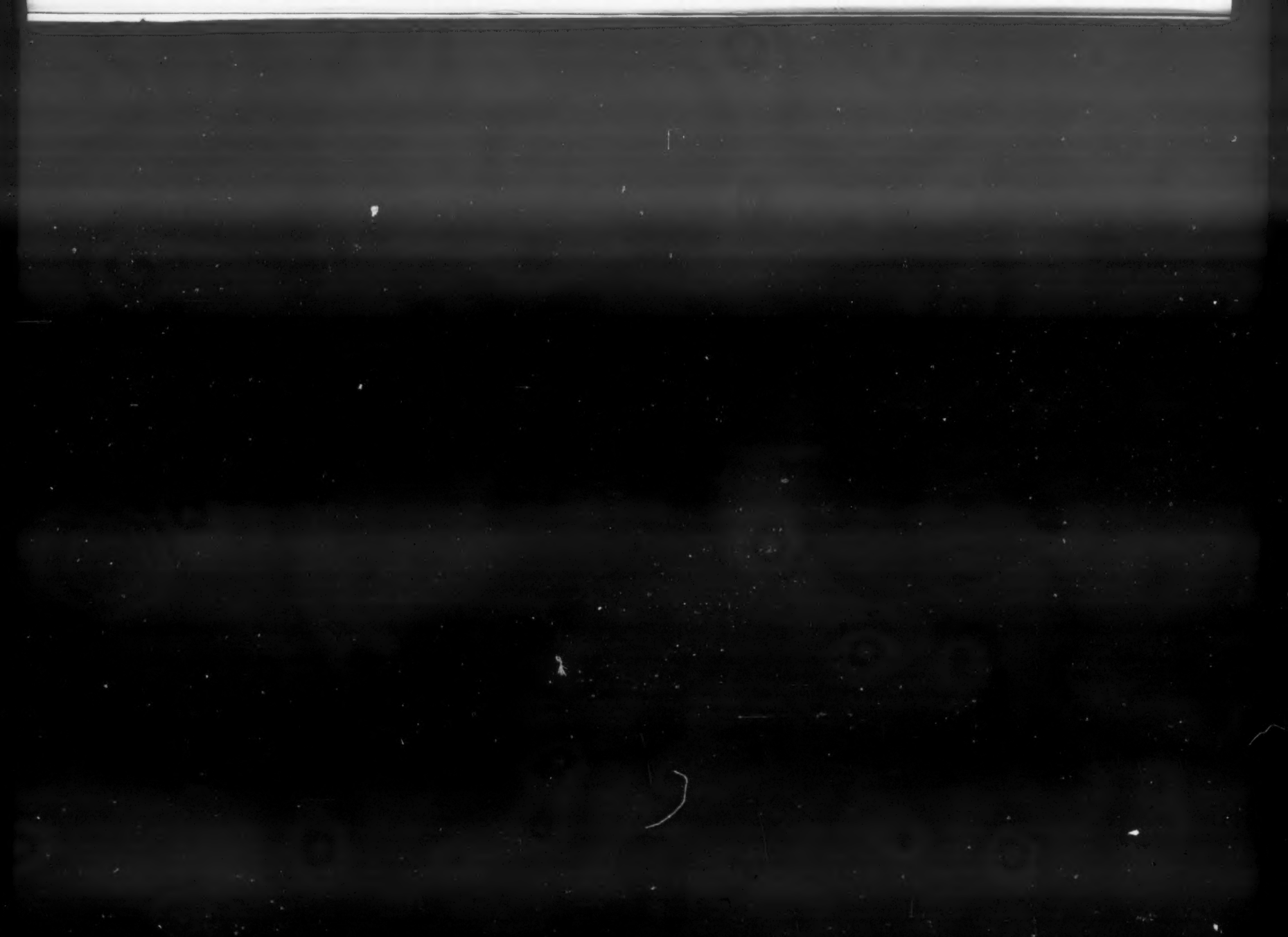
Symposium on the Normal Cervix (Cytology, Colposcopy, Colpomicroscopy, Histology)

Symposium on Cytology of the Large Intestine

Symposium on Fluorescence Microscopy

Symposium on the Significance and Interpretation of the Perinuclear Halo

Symposium on Cytology of Adenocarcinoma of the Corpus



# THE INTERNATIONAL ACADEMY OF GYNECOLOGICAL CYTOLOGY
# ACADEMIE INTERNATIONALE DE CYTOLOGIE GYNECOLOGIQUE
# INTERNATIONALE AKADEMIE FÜR GYNÄKOLOGISCHE ZYTOLOGIE
# ACADEMIA INTERNACIONAL DE CITOLOGIA GINECOLOGICA

FOUNDED IN BRUSSELS, BELGIUM, IN JULY, 1957


## HONORARY PRESIDENT

GEORGE N. PAPANICOLAOU

## EXECUTIVE COUNCIL

HANS KLAUS ZINSER, Cologne, West-Germany
*President*

J. PAUL PUNDEL, Luxembourg, Luxembourg
*President-Elect*

RUTH M. GRAHAM, Buffalo, New York, U.S.A.
*Secretary-Treasurer*

GEORGE L. WIED, Chicago, Illinois, U.S.A.
*Editor*

JEAN DE BRUX, Paris, France
CLARICE DO AMARAL FERREIRA, Rio de Janeiro, D.F., Brazil
JULIETA CALDERON DE LAGUNA, México, D.F., México


All communications pertaining to the International Academy of Gynecological Cytology and membership therein should be directed to the Office of the Secretary: 666 Elm Street, Buffalo 3, New York, U.S.A.

# INTER-SOCIETY CYTOLOGY COUNCIL

## OFFICERS

**CYRUS C. ERICKSON, Memphis, Tennessee**
***President***

**PAUL F. FLETCHER, St. Louis, Missouri**
***President-Elect***

**LEOPOLD G. KOSS, New York, New York**
***Vice-President (Cytology)***

**ROGER B. SCOTT, Cleveland, Ohio**
***Vice-President (Clinical)***

**EMMERICH VON HAAM, Columbus, Ohio**
***Vice-President (Pathology)***

**PAUL A. YOUNGE, Brookline, Massachusetts**
***Secretary-Treasurer***

**JOHN F. SEYBOLT, New York, New York**
***Assistant Secretary***

## EXECUTIVE COMMITTEE

| *Cytology:* | *Clinical:* | *Pathology:* |
|---|---|---|
| **GEORGE L. WIED**<br>**Chicago, Illinois (1962)** | **HAROLD C. MACK**<br>**Detroit, Michigan (1962)** | **JAMES W. REAGAN**<br>**Cleveland, Ohio (1962)** |
| **JOHN K. FROST**<br>**Baltimore, Maryland (1961)** | **SAUL B. GUSBERG**<br>**New York, New York (1961)** | **WILLIAM M. CHRISTOPHERSON**<br>**Louisville, Kentucky (1961)** |

**ABRAHAM E. RAKOFF**
***Past President***

**All communications concerning the Inter-Society Cytology Council should be directed to the Office of the Secretary-Treasurer: 1101 Beacon Street, Brookline 46, Massachusetts, U.S.A.**

# SOCIEDADE BRASILEIRA DE CITOLOGIA

FUNDADA NO RIO DE JANEIRO A 1º DE SETEMBRO DE 1956

**PROF. DR. ARNALDO DE MORAES**
*Presidente Honorário*

**DOC. DRA. CLARICE AMARAL FERREIRA**
*Presidente*

**DOC. DR. MÁRIO BENNING KAMNITZER**
*Vice-Presidente*

**DRA. ILKA MENEZES**
*Secretário Geral*

**DR. GEORG SCHNEIDER**
*Secretario de atas*

**DOC. DR. NÍSIO M. FONSECA**
*Tesoureiro*

**DOC. DR. ANTONIO A. QUINET**
*Redator de publicações*

**DOC. DR. ANTONIO VESPASIANO RAMOS**
**DOC. DR. MÁRIO PARDAL**
**DR. ADRIANO C. FERREIRA**
*Comissão de Admissão*

**All communications pertaining to the Brazilian Cytology Society and membership therein should be directed to the Office of the President: Emilio Berla 46, 3.° andar, Copacabana, Rio de Janeiro, Brasil.**

# SOCIETÀ ITALIANA DI CITOLOGIA CLINICA E SOCIALE

*Comitato Nazionale:*

**PROF. MARIO TORTORA**
*Clinica Ostetrica e Ginecologica Università, Sassari*
*Presidente*

**PROF. LUIGI CUSMANO**
*Ostetrico Ginecologo Ospedale Maggiore, Novara*
*Segretario*

**PROF. PRISCO NATALE**
*Ginecologo Instituto Nazionale Cura Tumori, Milano*
*Tesoriere*

**SOCI FONDATORI:**

**Luigi Bacialli**
**Claudio Barigozzi**
**Brunetti Fausto**
**Raffaele Calvanico**
**Luigi Cattaneo**
**Mario Cetroni**
**Carlo Chiaudano**
**Luigi Cusmano**
**Ettore De Biasi**
**G. Federico De Gaetani**
**Giuseppe Dellepiane**
**Wladimiro Inglulla**
**Massimo Macciotta**
**Piero Malcovati**
**Franco Marchesi**
**Mario Massazza**
**Eugenio Maurizio**
**Alberto Midana**
**Angelo Migliavacca**
**Espedito Moracci**
**Giuseppe Motta**
**Giacomo Mottura**
**Prisco Natale**
**Giovanni Paroli**
**Pietro Quinto**
**Giovanni Revoltella**
**Dino Roccia**
**Giuseppe Rossi**
**Salvatore Scaglione**
**Carlo Sirtori**
**Pompeo Spoto**
**Giuseppe Tesauro**
**Mario Tortora**
**Giuseppe Valle**
**Francesco Vozza**

**All communications pertaining to the Società Italiana di Citologia Clinica e Sociale should be directed to the following address: Segretaria, Società Italiana de Citologia Clinica e Sociale, Istituto S. Giuliano, Baluardo Lamarmora 10, Novara, Italy.**

# ASOCIACION MEXICANA DE CITOLOGIA EXFOLIATIVA

**DRA. JULIETA C. DE LAGUNA**
*Presidente*

**DR. ALEXANDER MEISELS**
*Vice-Presidente*

**DRA. BLANCA I. CONDE**
*Secretario*

**DR. MARIO GONZÁLEZ RAMOS**
*Tesorero*

**All communications pertaining to the Mexican Association of Exfoliative Cytology should be directed to the following address: Dra. Blanca I. Conde, Graciela 135, Mexico 14, D.F., Mexico.**

# BUSINESS MATTERS
# OF THE INTERNATIONAL ACADEMY

## FROM THE OFFICE OF THE PRESIDENT

In accordance with the Bylaws of the International Academy Article I, Section 3, the

### FIRST INTERNATIONAL CONGRESS OF EXFOLIATIVE CYTOLOGY

is scheduled for August 31 to September 2, 1961, immediately preceding the Third World Gynecology Congress. The place of the meeting will be Vienna, Austria.

*Extracts from the Bylaws concerning the Scientific Session* (*Article 1, Section 10*) : ". . . Papers presented at the Scientific Session shall be original papers which have never been presented or published. Material which has already been published or presented elsewhere may be considered in panel discussions. Fifty per cent of the Scientific Session shall be devoted to original papers, and fifty per cent of the time to panel discussions. . . ."

The attendance and participation is *not* restricted to members of the International Academy.

The Congress of Exfoliative Cytology will deal with, among other general topics, the following two main subjects:

(1) Progression and regression of epithelial abnormalities, and

(2) The hormonal activity as reflected in the vaginal smears in cases of gynecological tumors, breast tumors, and ovarian dystrophy.

In addition to other panel discussions, there will be an extensive discussion on cytological and histological terminologies and definitions.

Application forms for participants may be obtained from

The Office of the Secretary-General
First International Congress of Exfoliative Cytology
666 Elm Street
Buffalo 3, New York, U.S.A.

Made in West Germany

*Extremely versatile*

# RESEARCH MICROSCOPE

CARL ZEISS

**MODEL WL**

The latest Carl Zeiss advancements in microscope design are embodied in this instrument, making it ideally suited to the needs of modern science. It is outstanding in optical performance, quick setting, and ease of operation.

The underlying principle of its design permits quick interchangeability of parts. It is, therefore, readily adaptable for special tasks.

The WL is well suited for microscopic observation in brightfield, darkfield, phase contrast, polarized light, as well as incident light.

*Write for detailed literature*

**CARL ZEISS, INC.**

485 FIFTH AVENUE, NEW YORK 17, N. Y.

COMPLETE SERVICE FACILITIES

TIMESAVING * ACCURATE * INEXPENSIVE

# Simple Object Marker for Cytological Smears

- Puts small uniform rings on the slides
- Identifies the individual cytotechnician by usage of different colors of ink
- Is easy to handle
- Will not break glass slides when marking them
- Comes equipped as a complete kit with ink, cleaner, pad and object marker
- Is in paracentral alignment with the objective

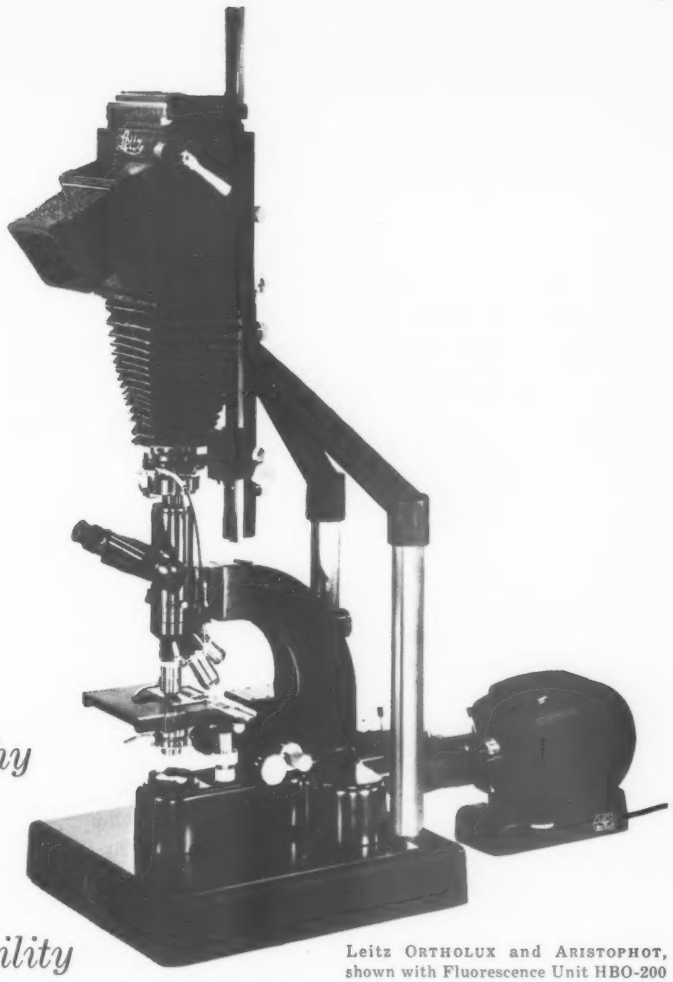
The object marker is attached to the microscopic nosepiece in the same manner as a regular objective. When the cytotechnician has found the area on the specimen which requires further attention, she inks the marker and then rotates the microscopic nosepiece to the place of the objective. A slight pull on the cylindrical part of the object marker stamps a ring mark on the slide.

*For further details write to:*

**ZIELER INSTRUMENT COMPANY**

**31 St. James Avenue**

**BOSTON 16 · MASSACHUSETTS · U.S.A.**



*photomicrography*
*combining*
**LEITZ** *quality*
*with*
*unlimited versatility*

Leitz ORTHOLUX and ARISTOPHOT,
shown with Fluorescence Unit HBO-200

# ORTHOLUX *Microscope with* ARISTOPHOT II

Here are precision Leitz instruments perfectly matched for exacting photomicrography. They are simple to operate, yet capable of infinite variations to fit all your photomicrographic requirements.

With the Leitz ORTHOLUX with ARISTOPHOT, image sharpness is limited only by the resolution of the film you are using. Only air separates the optical system from the film. There is no loss of image sharpness because of mirrors or other devices.

Film size is not restricted: 35mm Leica, 3¼ x 4¼, 4 x 5, or Polaroid. Your choice of illumination, too, is virtually unlimited: transmitted or incident light, dark field, polarized or phase contrast.

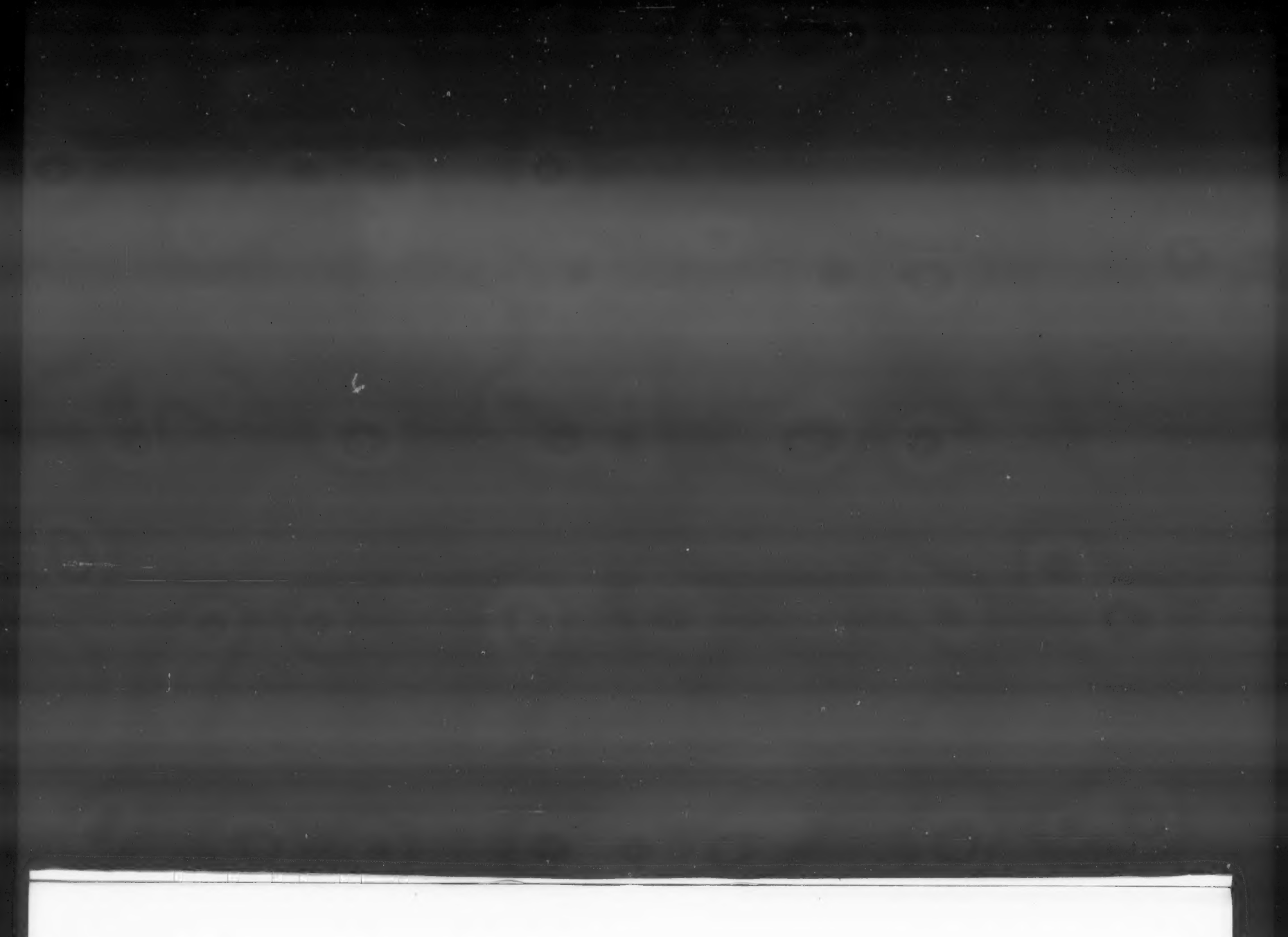
With the ARISTOPHOT detached, the Leitz ORTHOLUX fulfills the most critical requirements for a research microscope. The ARISTOPHOT II may be used separately for gross photography or macrophotography.

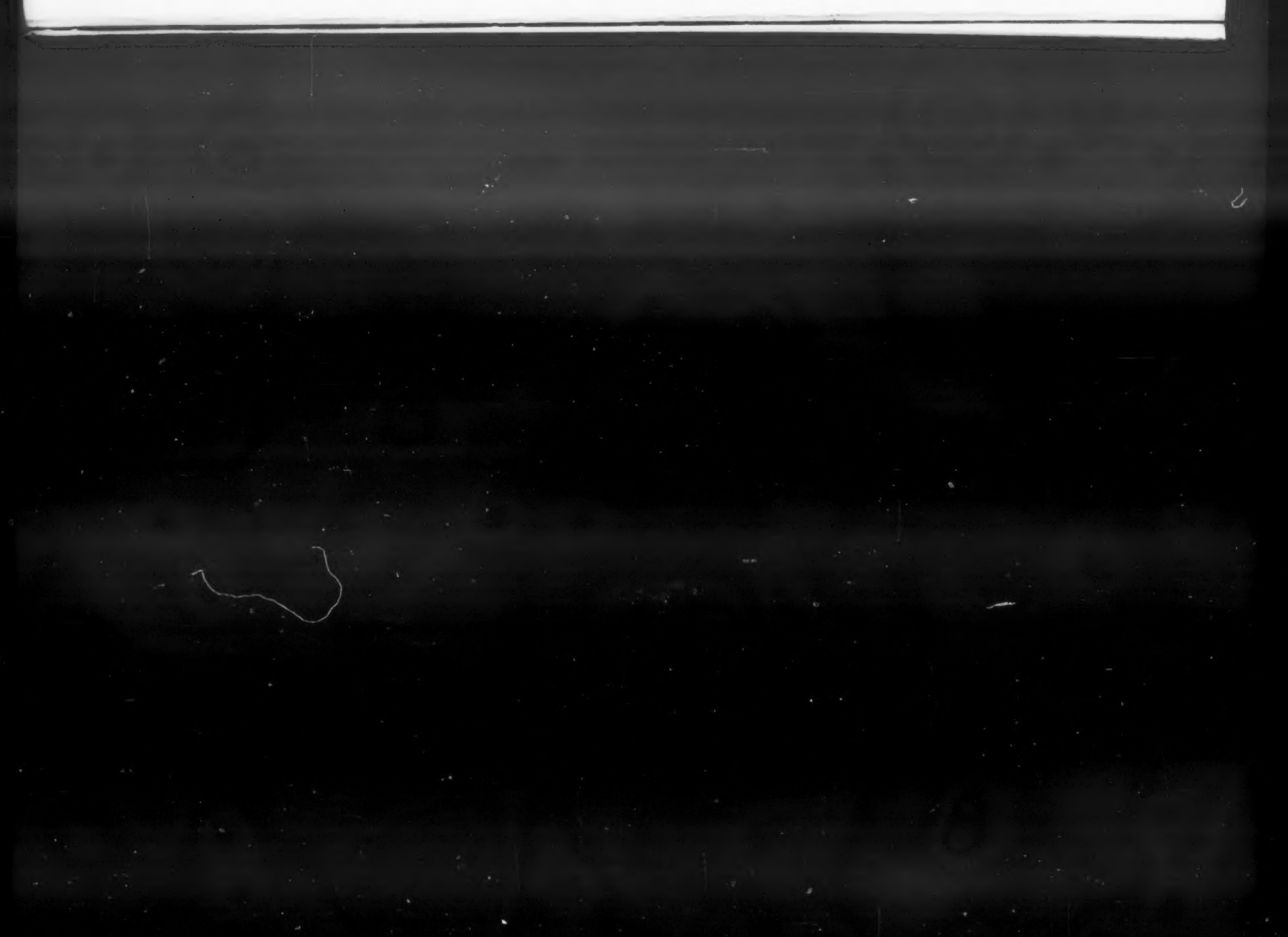
Write for illustrated brochure, Dept. 294.

Leitz

E. LEITZ, INC., 468 PARK AVENUE SOUTH, NEW YORK 16, N. Y.
Distributors of the world-famous products of
Ernst Leitz G. m. b. H., Wetzlar, Germany–Ernst Leitz Canada Ltd.
LEICA CAMERAS · LENSES · PROJECTORS · MICROSCOPES · BINOCULARS

29459





#254